Understanding the relationships between short-term feeding behaviour and long-term intake

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DEDICATION

This work is dedicated to my family for their support and encouragement and especially my mother who sadly did not live to see me complete this challenge.

"Doubly happy, however, is the man to whom lofty mountain-tops are within reach"

John Muir

ABSTRACT

The prediction of animal performance under a variety of conditions has long been a goal within animal science. Because animal performance is a function of food intake this has to be predicted if performance is to be effectively modelled. Analysis of short-term feeding behaviour may give insight into the regulation of feed intake allowing better models of intake to be constructed. This thesis examines in detail the short-term feeding behaviour of dairy cows to better understand food intake regulation.

Feeding behaviour consists of feeding events separated by non-feeding intervals. Feeding events are generally clustered into meals. Chapter 2 describes a methodology that utilises a combination of Gaussian and Weibull distributions to describe the length of intervals between feeding events. This method estimates a meal criterion, which allowes the clustering of feeding events into meals in a biologically acceptable way. In subsequent chapters short-term feeding behaviour is assessed largely in terms of meals.

Changes in the probability of animals starting a meal with time since the last meal is thought to give insight into the mechanisms underlying intake regulation. Starting probabilities are often calculated with data pooled across individuals or day and night. The extent to which pooling affects conclusions was assessed by analysing experimental data from cows. Results were used to parameterise simulation models, where the consequences of data pooling were systematically investigated. This

showed that as variability in pooled data increases so did the likelihood of misinterpreting results.

When offered a choice of foods, animals are able to select a consistent combination of these foods in the long-term. Analysis of how a consistent diet is achieved may give insights into the mechanisms regulating intake. The hypothesis that consistent long-term diet choice may be a consequence of diet selection within meals was tested using probability theory. This showed that cows did not have more meals with a composition that was similar to the long-term average diet choice than expected by chance. Therefore, no evidence was found to support such short-term regulation of diet choice.

Analysis in previous chapters suggested methods for exploring short-term feeding behaviour, which had yet to be attempted. These included calculation of changes in the probability of animals ending a meal with time since the start of the meal, diurnal patterns of diet choice and prandial correlations. The hypothesis that such analyses could give insights into mechanisms controlling feeding behaviour was tested. No evidence of food intake or diet choice regulation in the very short-term was found.

The final chapter examined previously published theories of intake regulation. Predictions from these theories were contrasted with the findings presented in this thesis and in the literature. Theories that advocate regulation in the short-term, and other theories, are identified and discussed in light of current findings. The direction of future work, to enable successful prediction of intake, is discussed.

DECLARATION

I hereby declare that this thesis is of my own composition and that all assistance has been duly acknowledged. The results presented herein have not previously been admitted for any other degree or qualification.

Martin Paul Yeates

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TABLE OF CONTENTS

CHAPTER ONE

Understanding the relationship between short-term feeding behaviour and long-term feed intake

1.1. General introduction

The ability to predict the food intake of animals, both within natural and domestic environments has long been of interest to man (Forbes, 1985). Within the ecological sciences emphasis has been on understanding the effects of foraging on both the natural environment and on lifetime fitness, in terms of survival and reproduction (Newman *et al.*, 1995; Illius *et al.*, 1999; Shipley *et al.*, 1999). In contrast, the principal interest to animal scientists has been the prediction of food intake, because this largely determines animal performance, e.g. meat or milk production, which has huge economic implications (Ketelaars and Tolkamp, 1992a; Poppi *et al.*, 1994; Weston, 1996; Forbes, 2000).

For some production systems the performance of animals has been predicted with food intake as a known input, i.e. where animals are fed a given amount and consume all available food (Kristensen, 1986; Mertens, 1996; Emmans and Kyriazakis, 2001). However, the more common situation, especially for ruminants, is that of *ad libitum* food intake; this increases the complexity of the problem. The ability to predict the intake of *ad libitum* fed animals would allow appropriate foods to be supplied to animals in correct quantities, thus avoiding waste, maintaining health and welfare and maximising efficiency of production. Therefore, accurate prediction of food intake continues to be a subject of great importance to both animal and ecological scientists (Forbes, 1977a; Forbes, 1977b; Belovsky, 1978; Schilstra, 1981; Mertens, 1987; Illius and Gordon, 1991; Ketelaars and Tolkamp, 1992a; Gill and Romney, 1994; Fisher, 1996; Mertens, 1996; Poppi, 1996; Emmans, 1997; Day

et al., 1998; Illius *et al.*, 1999; Pittroff and Kothmann, 1999; Shipley *et al.*, 1999; Yearsley *et al.*, 2001; Fisher, 2002)

Historically, the favoured approach to the prediction of food intake has been to assume that intake in a given environment will be repeatable over time. Therefore, multiple regression models, that use past events as predictors of future food intake, have been widely used within animal science to predict daily food intake and consequently animal production (see Forbes, 1995a). This empirical approach has been advocated, for instance, by the Agricultural and Food Research Council (AFRC, 1993) where, for dairy cows, daily dry matter intake is predicted to be a function of the weight of the animal, its milk yield, the week of lactation and the amount of concentrated food in the diet. Such models can predict intake with acceptable accuracy, if used within the range of conditions for which they were parameterised. However, their use outside of the conditions over which they have been generated will be of limited value (Kyriazakis and Emmans, 1999). This was well demonstrated by Forbes (1983) where it was shown that the regression equation of Vadiveloo and Holmes (1979) predicted the dry matter intake of a 275kg non-lactating heifer to be 0kg per day when their equation was extended beyond its parameterisation range. Therefore, regression equations are purely descriptive empirical models that make no attempt to understand the biological processes that animals use to control their food intake.

Models that attempt to predict intake, based on an understanding of how animals regulate the quantity of food they consume, may therefore offer an alternative

approach (Poppi *et al.*, 1994; Kyriazakis and Emmans, 1999). To construct such models it is important to carefully consider the basis of intake control mechanisms (Weston, 1996). Indeed, throughout evolution animals have lived in environments where *ad libitum* intake of food was often possible, therefore it would have been necessary to make choices about which foods to consume and when to stop eating. Animals are therefore likely to have developed rules or mechanisms, which enabled them to regulate their intake. If such mechanisms did not exist then animals would often be observed to either not eat when food was available and hence starve, or conversely, suffer the negative consequences of continuous over consumption, e.g. digestive disorders and the inability to escape predators (Weston and Poppi, 1987; Mertens, 1996). The ability of animals to regulate their intake is therefore likely to have developed to ensure survival and enhance fitness (Illius *et al.*, 2002).

If mechanisms that enabled animals to regulate intake were genetically controlled, at least in part, and these mechanisms conveyed an advantage to animals with the necessary genetic composition then such animals may have been more likely to reproduce than their conspecifics. Such animals could then be considered as more fit. The genes that helped animals to regulate their intake would therefore have increased within the population and as a consequence the ability to regulate intake would have been established.

If animals have developed the ability to regulate their intake then this is necessarily part of a strategy that aims to maximise fitness in the long-term, e.g. lifetime fitness (Illius *et al.*, 2002). However, animals are unlikely to be able to anticipate future

food availability with great accuracy and integrate this information with the digestive consequences of all previously eaten foods (Mertens, 1996). Therefore, it seems more plausible that animals would have developed a mechanism for intake regulation in the short-term (i.e. over a few hours for large herbivores) or medium-term (i.e. over a few days for large herbivores). Animals could then use the goal of such short or medium-term regulation mechanisms as a proxy for long-term fitness maximisation.

Regulation of short-term feeding behaviour would ensure that food intake is maintained at a consistent level in the short-term. Therefore, the long-term average intake would be the direct result of regulation of short-term feeding behaviour. Such short-term intake regulation could have direct benefits such as a regular supply of nutrients, which is thought by some to be important for optimum digestion and metabolism (Sinclair *et al.*, 1995; Kim *et al.*, 1999a; Witt *et al.*, 1999a), or to maintain sufficient energy levels (Le Magnen, 1985). However, an alternative feed intake regulation mechanism may have evolved whereby animals regulate their intake in the medium-term. Such an intake regulation strategy would allow animals to have flexible short-term feeding behaviour (e.g., Friggens *et al.*, 1998) but enable relatively consistent medium-term intake, i.e. when averaged over a day or more (e.g. Allcroft, *et al.*, 1999). Such animals could achieve a consistent medium-term intake via many different short-term feeding patterns.

The food that animals consume in the long–term must necessarily be a result of the feeding behaviour that occurs in the short-term (Forbes, 1985). Therefore, analysis of

short-term feeding behaviour has been advocated as a way of better understanding the intake regulation mechanisms used by animals (Sanderson and Vanderweele, 1975; Forbes, 1985; Dürst *et al.*, 1993; Dado and Allen, 1994; Gill and Romney, 1994). The question of regulation of feeding behaviour is the subject of this thesis. This is approached through novel analyses of short-term feeding behaviour. If strong evidence could be found that animals do indeed regulate their intake within the short-term then such analysis will provide the basis for prediction of intake directly from models of short-term feeding behaviour. Long-term intake would then be the direct consequence of short-term feeding behaviour.

If short-term feeding behaviour is to be analysed to explore the question of how animals regulate their intake then it is important to address a number of methodological issues related to short-term feeding behaviour analysis. This serves two purposes. First, by exploring the implicit assumptions of current models (e.g., Slater and Lester, 1982; Sibly *et al.*, 1990; Berdoy, 1993; Tolkamp *et al.*, 1998a; Tolkamp and Kyriazakis, 1999b; Forbes, 2001), which attempt to describe the short-term feeding behaviour of animals, it will be possible to determine if these models are appropriate for the analysis of animal feeding behaviour. Tolkamp *et al.* (1998a) have suggested that the current state regarding this is far from satisfactory. Only with models that fully capture the observed feeding behaviour can we attempt to better understand how animals regulate their intake. Therefore, if current models are found to be inadequate then the detailed analysis of short-term feeding behaviour will provide excellent information for the refinement of these methodologies. Secondly, it is important to ensure that any information, which can be gleaned from analysis of

short-term feeding behaviour, is not affected by inappropriate processing of experimental results (Panksepp, 1973; Morgan *et al.*, 2000b). Currently this is the subject of considerable debate. This methodological issue will therefore also be tackled. Substantial effort within this thesis has therefore been devoted to these issues.

To date, the efforts to understand the short-term feeding behaviour of animals have often focused on animals offered access to a single food (Slater, 1974; Le Magnen and Devos, 1980). However, in nature animals live in heterogeneous foraging environments, where they have to make choices about both the types and the quantities of foods to consume. Within animal science there has recently been interest in understanding how animals select a diet when supplied with a choice of foods (Kyriazakis and Oldham, 1993; Forbes and Shariatmadari, 1996; Provenza, 1996; Day *et al.*, 1998; Kyriazakis and Day, 1998; Pittroff and Kothmann, 1999; Tolkamp and Kyriazakis, 1999a; Forbes, 2001). Predicting the short-term feeding behaviour of animals in such conditions is even more challenging than when animals are supplied with a single food type only. As such, attempting to predict the diet that animals will select, and the short-term feeding behaviour they will exhibit, provides a severe test of theories which purport to describe the mechanisms animals use to regulate their intake and diet composition. For this reason, detailed analysis of the short-term feeding behaviour of animals offered access to more than one food as a choice will be conducted within this thesis. To that end, a new methodology for exploring short-term feeding behaviour of choice fed animals had to be developed.

For the purposes of detailed analysis of short-term feeding behaviour, which is conducted as part of this thesis, data were collected with dairy cows. The use of dairy cows has two principal attractions. First, cattle have a relatively slow metabolic rate and are therefore perhaps more suitable for analysis of whether intake is regulated over the short or medium-term than animals with faster metabolic rates (Simpson and Ludlow, 1986). Secondly, as discussed above, the predication of the *ad libitum* intake of ruminants has been of great interest for many years. As such, addressing the question of how animals regulate their intake by analysing the short-term feeding behaviour of dairy cows is particularly relevant.

The data used in this thesis was collected from cows that were offered access to a choice of two forage-based foods. Each food was formulated to have similar nutritional properties, except for their protein contents. The foods were supplied via computerised feeders that enabled very detailed short-term feeding behaviour data to be collected. Such data provide an excellent opportunity to gain insight into the mechanisms that animals use to regulate their intake and diet composition.

This thesis therefore addresses the following question, do animals regulate their short-term feeding behaviour to achieve a goal which is a proxy for long-term fitness maximisation? Alternatively, do animals aim for consistent food intake and diet composition in the medium-term and have largely flexible short-term feeding behaviour? Therefore, analyses presented within the thesis, which aims to better understand how animals regulate intake and diet composition, will be used to provide new evidence with which to explore current theories of intake regulation. If this

suggests animals regulate their feeding behaviour in a short-term deterministic way then theories in agreement will be highlighted. Alternatively, if short-term feeding behaviour is better described as probabilistic in nature then theories that indicate flexibility in the short-term will be supported. This analysis will provide new direction in the long running attempt to better predict the diet that animals consume.

1.2. Thesis structure

1. Develop a new methodology for describing short-term feeding behaviour, which overcomes weaknesses of current approaches and thus enables the short-term feeding behaviour of animals to be reliably assessed (Chapter 2).

2. Examine the issue of data pooling with respect to the analysis of short-term feeding behaviour. Develop novel simulation models to extend previous analysis. Highlight the current issues of concern and investigate when data pooling is appropriate (Chapter 3).

3. Use the information gathered in Chapters 2 and 3 to investigate the structure of feeding behaviour within the short-term when animals are offered a choice of two foods. Test the idea that animals select a diet of consistent composition within the short-term (Chapter 4).

4. Test ideas of short-term feeding behaviour regulation by analysis of feeding patterns and comparison with that expected if behaviour is regulated in the short-term (Chapter 5).

5. Explore the methodological developments within this thesis and their implications for previously published methodologies. Examine predictions of established theories of intake regulation using evidence gained from analysis of short-term feeding behaviour in Chapters 2-5. Investigate models where short-term feeding behaviour is used to predict animal behaviour and assess the appropriateness of this approach in light of the findings presented in this thesis. Suggest future work (Chapter 6).

CHAPTER TWO

The use of mixed distribution models to determine bout criteria for analysis of animal behaviour*

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2.1. Abstract

Feeding behaviour consists of feeding events, separated by non-feeding intervals. Feeding events are often clustered into bouts, which may be called meals. Grouping feeding events into meals requires the determination of a bout or meal criterion, that is, the longest interval accepted as part of a meal. Tolkamp and Kyriazakis (1999b) proposed a three-Gaussian model to estimate meal criteria. The three Gaussians each described the frequency distribution of the $\log_e$-transformed lengths of a population of intervals. These populations were thought to be: 1) short intervals within meals; 2) intervals within meals during which animals drink; 3) intervals between meals. This model predicted that the probability of an animal starting a meal would first increase, and then decrease, with time since the last meal. This contrasts with expectations based on the satiety concept, which predicts that the probability of an animal starting a meal will increase with time since the last meal. This discrepancy is related to the symmetrical nature of the Gaussian distribution. Alternatively, the two-parameter Weibull distribution can take a skewed form and perhaps is more suitable to describe the different populations of intervals. In this study, models consisting of combinations of Gaussian and Weibull distributions were examined for their suitability to describe observed feeding behaviour of cows. Weibulls did not improve the description of the populations of within meal intervals, compared to Gaussians. However, the Weibull distribution was found to describe the between-meal population of intervals statistically better than the Gaussian. Additionally, this inclusion of a Weibull, as opposed to a Gaussian, resulted in predictions that were in better agreement with the satiety concept over the entire range of interval lengths

observed. A model based on Gaussians to describe the within-meal populations of intervals and a Weibull to describe the population of between-meal intervals is, therefore, proposed. This model leads to biologically more satisfactory estimates of bout criteria than previous models and is likely to be applicable both across species and behaviours.

2.2. Introduction

The feeding behaviour of animals can be recorded in terms of events such as bites or visits to a feeder (Slater, 1974; Mayes and Duncan, 1986; Berdoy, 1993; Nielsen, 1999). These feeding events can be the unit in which feeding behaviour is analysed (Nielsen *et al.*, 1995; Davis, 1996). However, the definition of a feeding event is affected by the methodology used to collect data and the accuracy of the measurements (Tolkamp *et al*, 2000). This makes it problematic to compare studies of feeding behaviour that use different methodologies. In addition it has been questioned whether these short feeding events are the most relevant unit in which feeding behaviour is organised. It has been suggested that the meal, rather than the feeding event, would be a more relevant unit of animal feeding behaviour (Forbes, 1985; Sibly, *et al.*, 1990; Demaria-Pesce and Nicolaïdis, 1998; Barrio *et al.*, 2000; Tolkamp *et al.*, 2000).

The meal is a cluster of feeding events separated by short intervals (Mayes and Duncan, 1986), which can be distinguished from the next meal by a non-feeding interval that is long compared to the intervals within a meal (Sibly, *et al.*, 1990). If the meal is the unit of feeding behaviour that is of interest, then a meal criterion must be determined (Bigelow and Houpt, 1988). A meal criterion is an estimate of the longest non-feeding interval that is considered to be part of a meal. If a meal criterion is estimated in a quantitative way, feeding events can be clustered into meals in a repeatable manner (Slater and Lester, 1982; Berdoy, 1993). This provides a standard

technique of grouping feeding events into meals which allows comparisons of feeding patterns within as well as between different experiments.

Tolkamp *et al.* (1998a) applied a double-Gaussian model, i.e. fitting a mixture of two normal distributions, to $\log_e$-transformed interval lengths between feeding events. The two populations of intervals are thought to describe the intervals within meals, and the intervals between meals. The double-Gaussian model was later improved upon by the addition of a third Gaussian distribution, thereby splitting the within meal intervals into two populations. This additional population was shown to describe the within meal intervals in which animals (cows) went to the drinking trough, thereby increasing the mean length of this population of intervals (Tolkamp and Kyriazakis, 1999b).

The three-Gaussian model was seen to describe the observed frequency distribution of intervals between feeding events much better than previous models did (Tolkamp and Kyriazakis, 1999b). However, weaknesses were also evident with this model. It was shown by Tolkamp and Kyriazakis (1999a) that, after back transformation to the real time scale, this model predicted a starting probability that first increased, then decreased with time since the last meal. This is a direct consequence of the use of Gaussian distributions, which always have a symmetrical form. This property of the model is in conflict with the expectations based on the satiety concept. Le Magnen (1985) defines satiety as "a passive state of no hunger. The termination of a meal, ... is the onset of satiety". Therefore, it is generally assumed that as satiety decreases

with time since the last meal, there will be a continuously increasing probability of the next meal starting (see Tolkamp *et al*, 1998a for further discussion).

The Weibull distribution (Johnson *et al.*, 1994) is an increasingly used (Gupta and Kundu, 1999; He and Fung, 1999) failure time model that is able to describe non-symmetrical frequency distributions. The failure time of a product, or individual, is the time until a given event has occurred for the product or individual (Kachman, 1999), e.g. Dürr *et al.* (1999) described the herd life of Holstein cows. This model has found applications in the analysis of feeding behaviour. Initially, Simpson and Ludlow (1986) used the Weibull model to examine the tendency of locusts to start a meal and more recently Davis (1996) used the model to describe the licking behaviour of rats. The Weibull distribution can take a two-parameter form with a fixed origin, or a three-parameter form that allows the starting point of the distribution to move from the origin to another point on the x-axis. This property has particular relevance for the description of data sets that contain a population of observations whose range does not encompass the origin, e.g. potentially the population of between meal intervals. This study describes the consequences of the use of Weibull, instead of Gaussian distributions, to describe the frequency distribution of the $\log_e$-transformed interval length between feeding events. The aim is to develop a model that not only fits the observations statistically well, but is in better agreement with the principles of the satiety concept than existing models, in order to develop a biologically satisfactory meal criterion.

2.3. Materials and Methods

2.3.1. General

The study is based upon data collected as part of two diet choice experiments, described by Tolkamp *et al.* (1998b) and Tolkamp and Kyriazakis (1999b). Materials and methods relevant to the present study, and common to both experiments, are summarised below.

2.3.1.1. Cows, housing and daily routine

The experiments took place at the Langhill Dairy Cattle Research Centre (Edinburgh, Scotland). Cows were kept in a yard for the duration of the experiment. They left the yard twice daily during milking, which lasted for up to 60 minutes (between 06:00-08:00 and between 16:00-17:30), and on rare occasions for management reasons such as foot trimming and pregnancy diagnosis. During these times cows had no access to the feeders.

2.3.1.2. Foods, food dispensers, and feeding regime

Cows received 0.5kg of parlour concentrates at each milking. Both main foods were a mixture of 70% grass silage and 30% concentrate on a fresh weight basis. The

composition of the concentrates differed such that two foods, with either a high (**H**) or low (**L**) crude protein (**CP**) content resulted (185 and 131 g of CP/kg DM respectively). Both foods were offered as a choice in computerised food dispensers (Insentec B.V., Marknesse, The Netherlands) as described by Tolkamp *et al.* (1998a). Each food dispenser consisted of a 160 l capacity bin, mounted on two load cells. Access to the bin was via a pneumatically operated gate, controlled by a computer. This operated in conjunction with an individual cow identification system that responded to the transponder that each cow wore around her neck. On entry to a food bin, the cow's identification number, the weight of the bin and the time was recorded. As the cow left the feeder, the gate closed and the time was again recorded. The gate remained shut for at least ten seconds to allow stabilisation of the food bin prior to re-weighing. Time was measured to the nearest second, and weight to the nearest 0.1kg.

Access to the feeders was continuous except during milking and between 08:00 and about 09:30 when food residues were removed from the bins and fresh mixed food supplied. In the morning approximately three-quarters of the daily food was offered, and the remaining food was added to the bins during the afternoon milking. The quantities of foods offered were calculated daily to allow at least 10% refusals. Cows were seen to select a diet that consisted of about two-thirds H and one-third L food. To equalise cow pressure per feeder some non-experimental cows had access to L food only (Tolkamp *et al.*, 1998b). Water was available *ad libitum* from two troughs situated one near each end of the row of food dispensers.

2.3.2. Experiment 1

Feeding event data were collected from 16 cows, as described by Tolkamp *et al.* (1998b). All cows had access to 12 food dispensers, half of which contained the H and half the L food. For the duration of the experimental period, housing, management, and feeding were kept consistent. The lengths of intervals, between visits to the feeders, were calculated for each cow as the time between the end of one visit and the start of the next.

The data set consisted of 2504 cow days (average 156.5, s.e. 12.9 days per cow). 79575 intervals between visits to the feeder were recorded, of which all $\log_e$-transformed intervals (in seconds) greater than zero (79386 intervals) were used in subsequent analysis.

2.3.3. Experiment 2

The drinking behaviour of 30 cows was observed, as previously reported by Tolkamp and Kyriazakis (1999b). Cows had access to 24 food dispensers, half of which contained the H and half the L food. In brief, water troughs were monitored on about 30 occasions for variable periods of time between 06:00 and 24:00. When a cow was observed drinking from a water trough its identification number and the time given by the electronic feeding system was recorded. This allowed the time at which she previously left a feeder, and subsequently entered a feeder, to be identified in the

visit record files. Therefore, this allowed the identification of intervals in which drinking occurred, which were not collected during Experiment 1.

Three hundred and thirty nine intervals (range 118 to 34740 seconds) in which drinking occurred were identified by direct observation of the cows and were used in subsequent analysis.

2.3.4. Model fitting

All combinations of Gaussian (**G**) and two-parameter Weibull (**W**) distribution models (see Appendix A for the probability density functions (**pdf**) (Everitt, 1998) of these two distributions) were investigated to determine which offered both a biologically and statistically good description of the observations. Both two and three population models were examined. The three-parameter Weibull distribution (Johnson *et al.*, 1994) was also fitted in combination with Gaussian distributions. The models were fitted to the $\log_e$-transformed interval lengths (expressed in seconds) between feeding events. The models were fitted using a Fortran 90 program (see Ellis *et al.*, 1994 for a description of the programming language) which utilised double precision calculations throughout. The Fortran program utilised the NAG library routine E04JAF (NAG, 1993). Routine E04JAF performs an iterative process to find maximum likelihood estimates for the parameters of a model (Langton *et al.*, 1995).

Preliminary analysis demonstrated that, under these experimental conditions, the Gaussian distribution always described the first population of observed intervals significantly better than the Weibull distribution. Therefore, the more suitable Gaussian distribution was used throughout this study, to describe the first population of intervals. Only the differences in fit of the two proposed distributions, to the second and third populations, are therefore reported. The three-parameter Weibull model (Johnson *et al.*, 1994), which allows the origin of the distribution to move from zero, was also examined during preliminary analysis. The addition of a third parameter did not significantly improve the description of the observations, when compared to the two-parameter Weibull. Therefore, during all subsequent analysis two-parameter Weibull models were used.

2.3.5. Three-population models

Three-population models have been found to be appropriate for most dairy cows (Tolkamp and Kyriazakis, 1999b). These populations represent different types of intervals between feeding events. The populations are separated into short, within meal intervals, and long, between meal intervals. The short intervals are further separated into intervals during which cows are assumed to, or assumed not to, engage in drinking. Therefore, three-population models were initially fitted to the pooled observations. This allowed the best models to be identified before they were investigated further on the smaller data sets from individual animals.

2.3.5.1. *Fitting models to the pooled data*

The following three-population models; G-G-G, G-G-W, G-W-G and G-W-W, were fitted to the pooled observations from Experiment 1. Models with a Weibull distribution describing the third population of intervals between feeding events were investigated further. Models G-W and W-W were fitted to the observed intervals that contained drinking, from Experiment 2. These models represented the final two populations of models G-G-W and G-W-W, respectively. The first population was not observed, by definition, when looking for intervals that included drinking.

2.3.5.2. *Fitting models to the observations from individual cows in Experiment 1*

Model G-G-W was compared to model G-G-G for its fit to individual data from Experiment 1. The presence of a population of intervals, that are thought to represent intervals during meals in which drinking occurred (Tolkamp and Kyriazakis, 1999b), was assessed by visual inspection of the observed pdf and the fit of the models to the observations. The goodness of fit of these models was tested for those individual cows that showed evidence of having three populations of intervals. Model G-G-W was also fitted to the pooled data of those cows that showed evidence of three populations of intervals.

2.3.6. Two-population models

Two-population models (G-W and G-G) simply describe intervals that occur within, and between, meals. They were tested for their fit to the observations of individual cows from Experiment 1 that did not show evidence of drinking within meals. Model G-W was also fitted to the pooled observations of those cows that did not show evidence of having three populations of intervals.

2.3.7. Model testing

The quality of fit of the models to the observations was tested by examination of the graphical fit of the models and by statistical analysis. The statistical analysis was based on the likelihood values for the models. These are given by the maximum likelihood procedure in the Fortran program. The higher the likelihood value the better the model fits the data (Langton *et al.*, 1995). Because likelihood values are very small, they are often expressed as minimum function values (**MFV**). These are equivalent to twice the negative log-likelihood.

Tests were performed to determine if the differences in MFV between the models were significant. If the models being compared were nested (where a model, with n parameters, is completely included into a larger model, with > n parameters) then a likelihood ratio test was used. This assumed a Chi-Square distribution of the test

statistic (Kleinbaum *et al.*, 1988). All other models were compared using Akaike's Criterion (Armitage and Colton, 1998), where the likelihood values of models are directly compared, allowing for differences in the number of parameters. For models with the same number of parameters the smaller MFV (greater likelihood) signifies a better fit of the model to the data (Armitage and Colton, 1998). Thus when comparing the MFV of such models the MFV of one model may be set to zero relative to the other models tested. This enables the magnitude of the difference in MFV to be compared. It must be noted that the magnitude of these differences in MFV cannot be assigned a formal level of significance. However, according to Akaike's Criterion (Armitage and Colton, 1998) the larger the difference in MFV, the greater is the improvement in the statistical fit of the model.

The MFV was used to assess the statistical fit of models to the observations from individual cows. The differences in MFV between models, for each individual cow, were normalised by a signed square-root transformation (any negative values were multiplied by –1, square-rooted, then multiplied by –1 again). The transformed values were t-tested against a mean difference in MFV of zero, for all individuals.

Medians for each population of intervals were calculated from the estimated parameters of the models (see Appendix A) and then back-transformed to the real time scale by exponentiation.

2.3.8. Meal criterion

Meal criteria were calculated, from the parameters of the models, as the point at which the pdf of the final two populations cross. This minimises the mis-assignment of intervals to the wrong populations (Tolkamp and Kyriazakis, 1999b) and is, therefore, the preferred meal criterion (Slater and Lester, 1982). Models containing a Weibull distribution do not solve directly for t (see Appendix A). The meal criterion was therefore estimated using the Newton-Raphson iterative process (Stewart, 1986; Weltner *et al.*, 1986).

2.3.9. Starting probabilities

The observed starting probabilities were calculated as the number of intervals that had a length of between z seconds and $z + x$ seconds, divided by the number of intervals longer than z seconds. Probabilities were calculated for x = 5, 60 or 900-second intervals, for short, medium and long interval lengths respectively, to give a similar number of calculated probabilities for each distribution. The predicted starting probabilities for the same intervals (x) were calculated from the parameters of models G-G-G and G-G-W.

2.4. Results

2.4.1. Three-population models

2.4.1.1. The fit of models to the pooled data of Experiment 1

Table 2.1 demonstrates the fit of models G-G-G, G-G-W, G-W-G and G-W-W to the $\log_e$-transformed interval length between feeding events, pooled across cows, in Experiment 1. The fit of these models was not greatly affected by the choice of a Gaussian or a Weibull distribution to describe the second population. In contrast, the use of a Weibull to describe the third population of intervals had a marked effect on the fit of the models compared to model G-G-G. The Weibull distribution was found to take a left skewed shape for both the second and third populations (Figure 2.1). This resulted in the two models, with a Weibull distribution for the last population (G-G-W and G-W-W), describing the observations much better than the models with a Gaussian for this population.

Table 2.1 *The predictions of the given models when fitted to the log_e-transformed interval length between feeding events, pooled across cows, in Experiment 1*

Model	Minimum function value relative to G-G-G	Proportion of intervals in given population			Median of given population (min)			Estimated meal criterion (min)	Estimated number of meals/cow/day
		1st	2nd	3rd	1st	2nd	3rd		
G-G-G	0	0.57	0.26	0.17	0.62	2.27	189.36	49.5	5.59
G-G-W	-446	0.72	0.09	0.19	0.68	6.08	198.73	28.9	5.85
G-W-G	-9	0.50	0.32	0.18	0.64	1.64	189.36	28.0	5.86
G-W-W	-456	0.69	0.12	0.19	0.66	4.85	186.34	27.9	5.86

The proportion of intervals estimated to form part of the first population was lower, and the proportion of intervals estimated to be part of the second population was higher, for both models with a Gaussian describing the last population (Table 2.1). However, the proportion of intervals estimated to belong to the between-meal intervals was remarkably constant, ranging between 0.17 and 0.19. The meal criterion was seen to be much greater for the G-G-G model than the other three models. The higher meal criterion, for model G-G-G, resulted in a predicted number of meals per cow, per day, that was 0.26 lower than for model G-G-W.

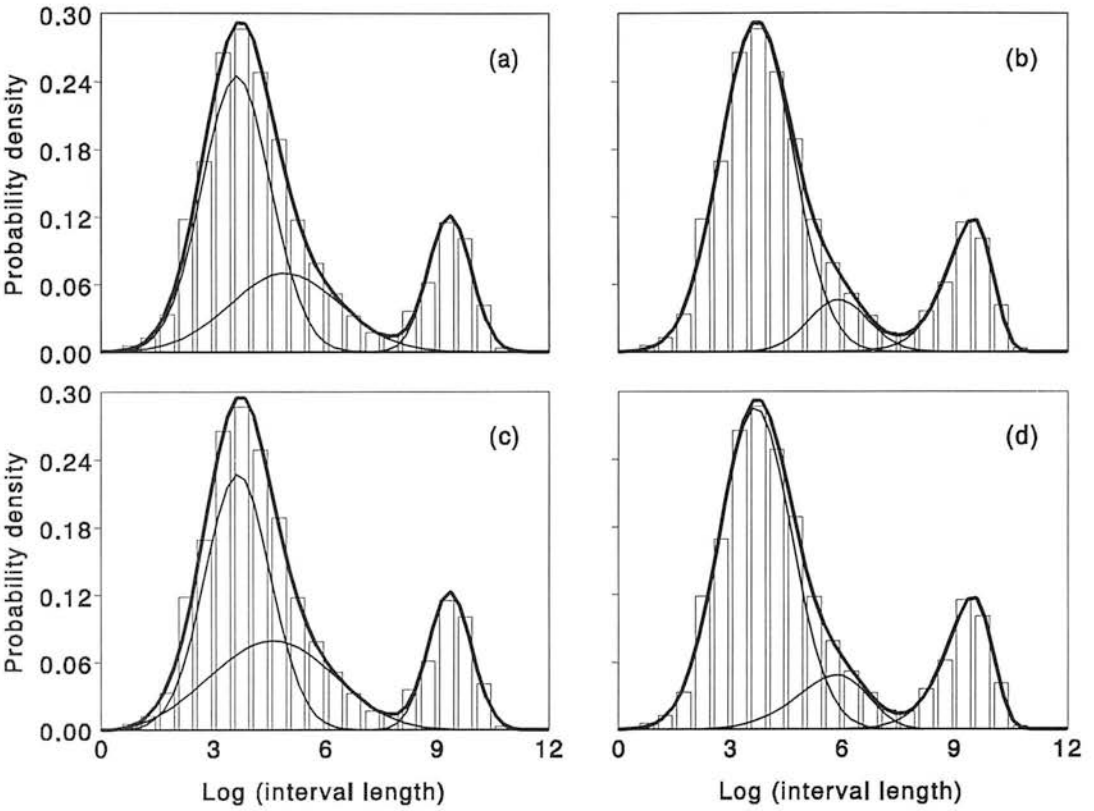

Figure 2.1 *The probability density function for models G-G-G, G-G-W, G-W-G and G-W-W (a, b, c and d respectively). Thin lines represent the contribution of each population to the overall probability density (thick line). The observed data, from Experiment 1, (relative frequency divided by class width, i.e. 0.5 $\log_e$ units) are represented by open bars.*

2.4.1.2. The fit of models to the observed drinking behaviour from Experiment 2

Models G-W and W-W, representing the final two populations of models G-G-W and G-W-W, were fitted to the pooled observations from Experiment 2. The

probability density function of these intervals is shown in Figure 2.2. Calculated medians, for the two populations, from model G-W was 7.79 and 154.46 minutes; compared to 8.21 and 153.77 minutes from model W-W. The model with a Gaussian distribution describing the intervals, within a meal, in which drinking occurred (G-W) gave a MFV that was 14 units lower than the W-W model and meal criteria of 28.7 and 26.9 minutes, respectively. Therefore, the model with a Weibull distribution describing the last population of intervals only, was statistically the better model.

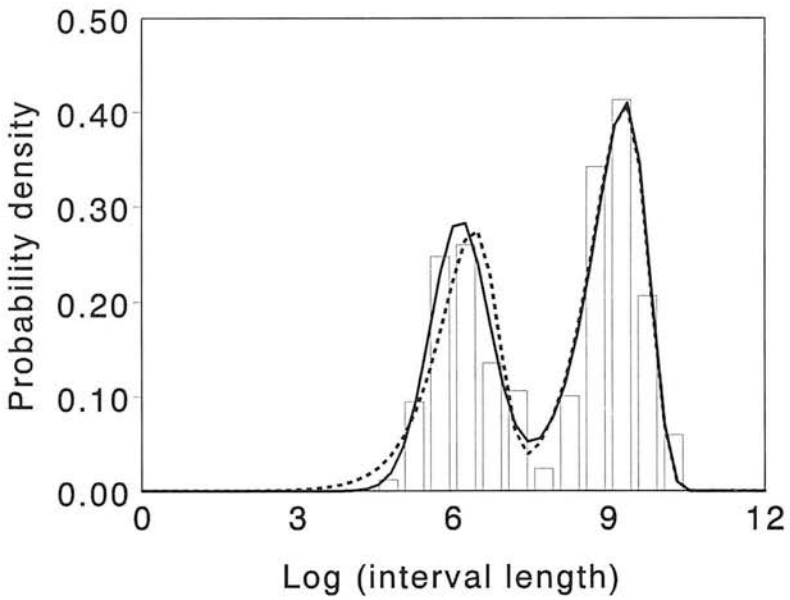

Figure 2.2. *The probability density function of models G-W (solid line) and W-W (broken line) fitted to the observations of intervals including drinking (relative frequency divided by class width, i.e. 0.5 $\log_e$ units) from Experiment 2 (open bars).*

2.4.1.3. *The fit of models to the observations from individual cows*

The fit of model G-G-W was assessed for individual cows from Experiment 1. This was compared to the fit of the model (G-G-G) proposed by Tolkamp and Kyriazakis (1999b). It was found that for 8 of the 16 cows three populations of intervals were evident. The statistical fit of the models to these eight individual cows were compared by examining differences in their MFV. Model G-G-W gave a significantly better fit ($t = 3.25$, $df = 7$, $P < 0.05$) to the observations of the eight individual cows than did model G-G-G. The estimates from model G-G-W, presented as mean±s.e. of the eight individuals, are given in Table 2.2.

Table 2.2. *Predictions (means±s.e.) from models G-G-W and G-W for individual cows with, and without, evidence for three populations of intervals, respectively.*

Parameter	G-G-W	G-W
Proportion of intervals in first population	0.70±0.02	0.80±0.02
Proportion of intervals in last population	0.20±0.02	0.20±0.02
Median of first population (minutes)	0.72±0.05	0.83±0.05
Median of second population (minutes)	8.00±1.39	
Median of third population (minutes)	188.30±13.00	185.27±7.57
Meal criteria (minutes)	29.0±2.24	22.2±0.99
Meals per day	6.05±0.36	5.94±0.22

Model G-G-W was also fitted to the pooled data of the eight cows that demonstrated three populations of intervals (Figure 2.3). Model G-G-W gave predicted proportions for the three populations as 0.72, 0.10 and 0.18, respectively. The meal criterion was estimated as 28.6 minutes. This resulted in an estimated 5.91 meals per cow per day.

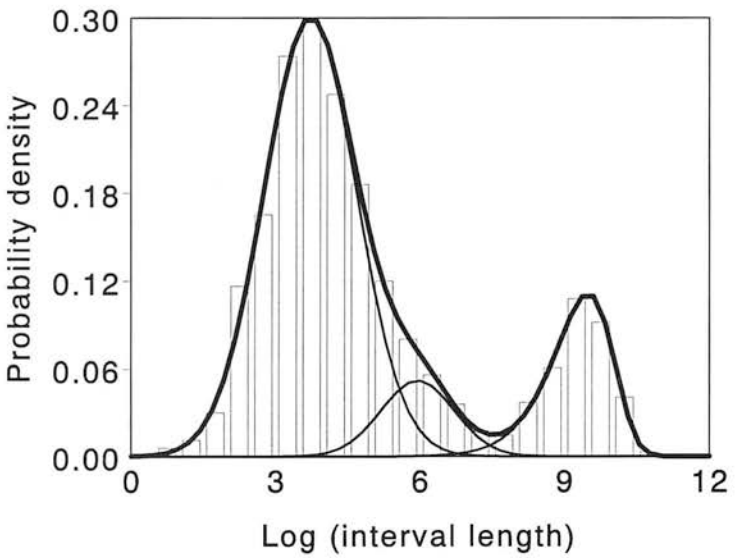

Figure 2.3. *The probability density function for the G-G-W model. Thin lines represent the contribution of each population to the overall probability density (thick line). The observed pooled data, from eight cows in Experiment 1 that demonstrated evidence of a third population of intervals, (relative frequency divided by class width, i.e. 0.5 $\log_e$ units) are represented by open bars.*

2.4.2. Two-population models

2.4.2.1. The fit of models to the observations of individual cows

The two-population models (G-G and G-W) were fitted to the individual data of the eight cows in Experiment 1 that did not show evidence of having a population of intervals corresponding to drinking within a meal. Model G-W was found to fit the individual cows significantly ($t = 4.20$, $df = 7$, $P < 0.005$) better than model G-G. The estimates from the model G-W, presented as means±s.e. of the eight individuals, are given in Table 2.2.

Model G-W was also fitted to the pooled data of the eight cows that did not demonstrate three populations of intervals (Figure 2.4). Model G-W gave predicted proportions of intervals in each of the two populations as 0.79 and 0.21, respectively. The meal criterion was estimated as 22.0 minutes. This resulted in an estimated 5.88 meals per cow per day.

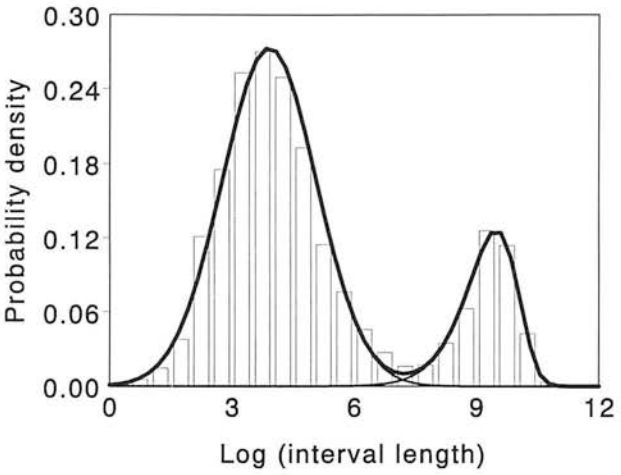

Figure 2.4. *The probability density function for the G-W model. Thin lines represent the contribution of each population to the overall probability density (thick line). The observed pooled data, (relative frequency divided by class width, i.e. 0.5 $\log_e$ units) from eight cows in Experiment 1 that did not demonstrate evidence of a third population of intervals, are represented by open bars.*

2.4.3. Starting probabilities

The starting probabilities predicted by models G-G-G and G-G-W were compared to the actual starting probabilities of the observations, pooled across cows, from Experiment 1. The starting probabilities predicted by these two models were almost identical for the within meal intervals. Therefore, only the predicted starting probabilities of model G-G-W are shown for the populations of short and medium interval lengths (Figure 2.5a and 2.5b). The starting probability, predicted by the models, can be seen to mirror the observations well. Figure 2.5c shows the starting probabilities, predicted from the two models, for the population of long intervals.

The G-G-G model does not describe the observations well. The starting probability predicted by the Weibull distribution increases in a way that accurately reflects the observed data.

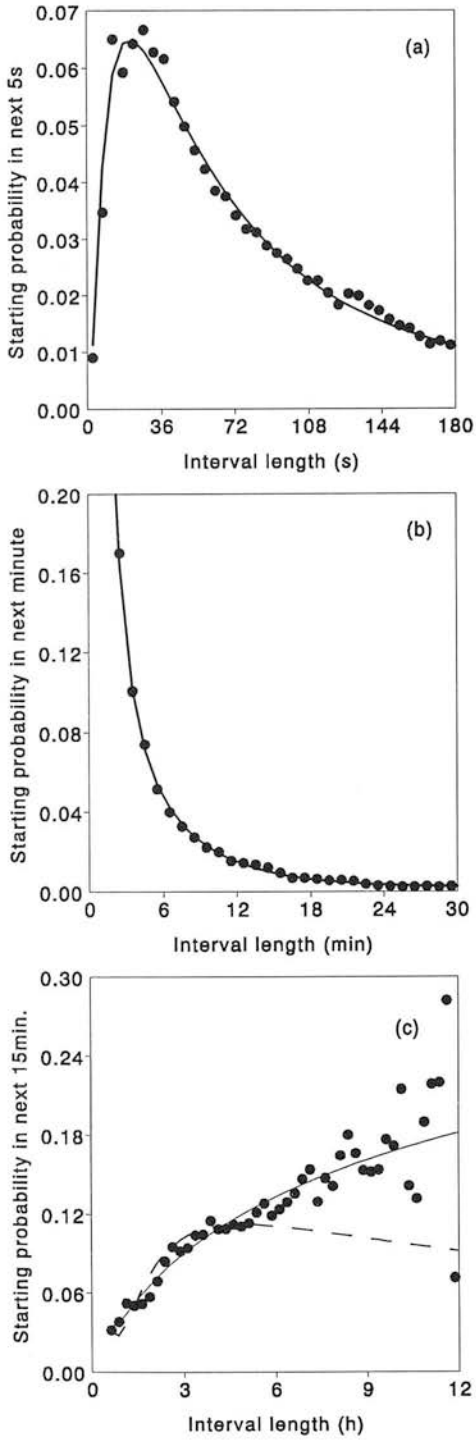

Figure 2.5. *Starting probabilities in relation to length of preceding, non-feeding, interval: (a) The probability that animals will start feeding in the next 5s, as observed (dots), and as predicted by model G-G-W (solid line). (b) The probability that animals will start feeding in the next 1 minute, as observed (dots), and as predicted by model G-G-W (solid line). (c) The probability that animals will start feeding in the next 15 minutes as observed (dots), and as predicted by model G-G-W (solid line) and G-G-G (broken line). The observations are those of the pooled data from Experiment 1. Predictions and observations shorter than 30 minutes.*

2.5. Discussion

Daily food intake is the sum of food intake during individual meals. Therefore, longer-term changes in intake can only result from changes in either size or frequency of meals, or both. Short-term feeding behaviour may, therefore, be of interest to those who wish to improve the understanding of the mechanisms of food intake regulation in the longer term (Dürst *et al.*, 1993; Mayes, and Duncan, 1986; Savory, 1999; Morgan *et al.*, 2000a). This knowledge could enable the development of more accurate food intake prediction models. If research in this area is to be fruitful then a standardised technique to cluster feeding events into meals that is applicable across experimental designs and methodologies is required. This study has investigated ways of grouping these intervals into populations in a way that allows a biologically relevant and statistically sound meal criterion to be estimated.

A model that included three-Gaussians to describe the frequency distribution of log-transformed interval lengths was a strong improvement on previous methodologies of meal criterion determination (Tolkamp and Kyriazakis, 1999b). Previous methodologies such as log-survivorship analysis (Slater and Lester, 1982) are based on the implicit assumption that the probability of animals starting meals is independent of time since the last meal. As a consequence they predict that there will be a high frequency of short intervals between meals. However, the satiety concept predicts that when an animal is satiated at the end of a meal, it is not likely to start feeding; this probability is expected to increase with time. As a consequence, there will be few short intervals between meals. The models developed by Tolkamp and

Kyriazakis (1999b) allowed for this. However, Tolkamp and Kyriazakis (1999a) later examined the predicted and observed starting probabilities when fitting their model to the observations from dairy cows. It was found that although observed and predicted starting probabilities coincided well for shorter intervals, they did not for very long intervals. The difference between the observed increasing starting probability and the predicted decreasing starting probability from the model, for intervals in excess of seven hours (see also Figure 2.5c), was thought to be the result of cow management procedures. This, however, could not be confirmed (Tolkamp, unpublished). Therefore, it seemed likely that the model could be improved upon by selecting a more biologically appropriate distribution.

Other previously published distributions were investigated to identify those that could provide an appropriate description of the non-feeding intervals. Distributions were examined for their suitability to describe the observed starting probabilities. The $\log_e$ of the observed starting probability was plotted against the $\log_e$ of the time scale and found the relationship to be approximately linear for long intervals. This property suggested the use of the Weibull to describe the frequency distribution of the $\log_e$ transformed interval lengths (Cox and Oakes, 1984). The Weibull distribution has a probability density function that can fit skewed data, in contrast to the symmetrical Gaussian. Additionally, the Weibull has the advantage, over other distributions (e.g. the Gamma, Johnson, *et al.*, 1994), that its probability density function, starting probability and median formulae all have user-friendly mathematical forms.

Because the distribution chosen to describe the population of long intervals can affect the fit of the distributions used to describe the other populations, all combinations of Gaussian and Weibull distributions were tested. The first population of observed intervals was always better described by a Gaussian than a Weibull distribution. The good fit of the Gaussian model to the within meal, non-drinking, intervals has previously been demonstrated (Tolkamp *et al.*, 1998a; Tolkamp and Kyriazakis, 1999b). This good fit is a product of the very skewed distribution of the first population of intervals when expressed on a real time scale. When the time scale of measurement is short compared with the time taken for an animal to move from one feeding event to another, few very short intervals can be expected, with a skewed distribution as a result (as discussed by Tolkamp, *et al.*, 1998a). Under such conditions $\log_e$ transformation then results in normalisation of the distribution.

When comparing models G-G-W and G-W-W, with G-G-G the improvement in fit was similar. To decide what the most appropriate model was to describe the population of drinking intervals, the models G-W and W-W were fitted to the observations from Experiment 2. The former model was clearly superior in its statistical description of the population of intervals. In addition, this model did not predict drinking intervals of less than two minutes. For animals to drink during a meal, under this experimental set up, they have to leave the food bin, move to the water trough and consume water, before returning to a feeder. Casual observations suggest this will take a period of time in the minutes range, thus very short intervals are not to be expected. Therefore, the model with a Gaussian describing the middle

population gave a statistically, as well as biologically, better description of the observations.

From the analysis it was evident that the use of a Weibull distribution to describe the population of intervals between meals was statistically superior to the use of a Gaussian (Table 2.1). However, the biological significance of such statistical superiority is not immediately clear. In addition, the superior fit may not be immediately evident from Figure 2.1. However, the biological implications of the two models are most clearly seen when the model parameters are used to predict starting probabilities (Figure 2.5c). In contrast with the starting probabilities predicted by the Gaussian distribution, those predicted by the Weibull continuously increased within the range of the observations. The latter is in agreement both with expectations based on the satiety concept and with observed starting probabilities (Figure 2.5c). Therefore, the inclusion of a Weibull distribution to describe the between meal intervals results in a model that describes the observation much better than the three-Gaussian model.

The proportions of intervals estimated, by the different models, to be in each population differed in a systematic way. Models containing a Weibull distribution describing the last population estimated a higher proportion of intervals in the first population and a lower proportion in the second. This is likely the result of the left-hand skew of the Weibull distribution which probably allowed the middle population to be smaller, therefore increasing the size of the first population. The G-G-G model was seen to give a meal criterion that was considerably higher than those given by

the other three models. However, as a consequence of the low number of intervals that were observed to be in the range of 20 – 50 minutes (corresponding to 7 – 8 $\log_e$ units, see Figure 2.1), this had a limited effect on the number of meals per day that were predicted by the models (range 5.59 to 5.86 meals/cow/day). Despite this, a methodology that better reflects the underlying biology is considered to be a fruitful step towards the understanding of how short term feeding behaviour is linked to longer term intake.

Pooling of data across cows is useful for identifying models that are applicable to the data. However, fitting models to individual cows allows the variation in fit between cows to be investigated. Therefore, the model was also fitted to observations from individual cows. It was found that the frequency distribution for some cows was better described by models with two, rather than three, populations. In both cases, a model that contained a Weibull rather than a Gaussian distribution for the last population was statistically superior. The estimated meal criteria, from model G-W, for the individual cows showing two populations, averaged 21.2 (s.e. = 0.09) minutes. This was significantly ($P < 0.05$) shorter than the average 29.0 (s.e. = 2.25) minutes predicted for cows showing three populations of intervals. Drinking within a meal will increase the length of the interval between feeding events. Therefore, the point at which the final two populations cross (the meal criterion) will be further from the origin of the x-axis; which results in longer estimated meal criterion for animals with three-populations of intervals. However, despite these significant differences in estimated meal criteria between cows that do or do not drink during

meals, the estimated number of meals per day, 6.05 (s.e. = 0.36) and 5.94 (s.e. = 0.22) were not found to be significantly different.

All animals eat their food in discrete meals rather than nibbling continuously (Forbes, 1985). It has been shown that many species have a characteristic lack of short intervals between meals, which can be seen as a flatter area on a log-survivorship curve (for a discussion of the similarity of these curves across species see Tolkamp *et al.*, 1998a). Different species exhibiting this range from cattle (Metz, 1975) and horses (Mayes and Duncan, 1986) to blowflies (Simpson *et al.*, 1989) and locusts (Simpson and Ludlow, 1986). The short term feeding behaviour observed in these studies seems to be in agreement with the prediction from the satiety concept. Therefore, a model based on this concept may have wider application than the species for which it was developed.

Behaviours other than feeding may also occur in bouts. Such bouts maybe separated by intervals in which the probability of the behaviour occurring changes with time since it last occurred. Examples of behaviours such as this are sexual behaviour and breathing patterns of intermittent breathers. Male rabbits (González-Mariscal *et al.*, 1997) and deer mice (Dewsbury, 1983) are seen to perform sexual behaviour in bouts until they are 'sexually satiated'. At this point the probability of further sexual behaviour is very low, but would be expected to increase with time. The breathing patterns of right whales (Winn *et al.*, 1995) and snapping turtles (Frische *et al.*, 2000) are further example of behaviours that occur in bouts. The length of the non-breathing interval between bouts seems also to be related to satiety like principles.

Frische *et al.* (2000) stated that the probability of a turtle commencing a bout of breathing increases with time since the last bout. This is similar to the changing probability of a meal starting, with time since the last meal. Frische *et al.* (2000) used the two-Gaussian model (Tolkamp *et al.*, 1998a) to describe the frequency distribution of $\log_e$ transformed interval lengths between breathing. This demonstrates that a model based more on satiety than random processes can be used to describe behaviours other than feeding.

In conclusion, the proposed model has been found to give a statistically and biologically better description of the feeding behaviour of cows when compared to the model of Tolkamp and Kyriazakis (1999b). The predicted probability of animals starting meals is now in agreement with both the satiety concept and the observations. The model can now be applied to cluster behavioural events into bouts, whenever the principle of satiety applies. This may include not only feeding behaviour across a wide range of species, but also other types of behaviour.

CHAPTER THREE

Consequences of variation in feeding behaviour for the probability of animals starting a meal as estimated from pooled data*

*This chapter has been adapted for publication as:

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3.1. Abstract

Better understanding of how animals regulate their intake may be gained by analysis of feeding behaviour. This is often recorded in terms of feeding events, e.g. visits to feeders, which can be clustered into meals. This enables calculation of the probability of animals starting a meal in relation to time since the last meal, which is thought to give insight into food intake regulation. Starting probabilities are often calculated with pooled data but recent work suggests that pooling may strongly affect conclusions.

In this study the feeding behaviour of cows was analysed to investigate how previous conclusions about feeding behaviour may have been affected by pooling. Using parameters derived from experimental data, simulation models were constructed to further explore under what circumstances pooling, either across day and night or across individuals, could affect the interpretation of starting probabilities. Data were simulated to explore the consequences of pooling as either the proportion of meals occurring during the day or the individual variation in their mean number of meals per 24 h changed. Simulation allowed the analysis of the consequences of pooling for the interpretation of starting probabilities to be extended.

Analysis of experimental data, collected with 16 dairy cows, showed that they ate a mean of six meals per 24 h. Individual variation resulted in a proportional CV of the individual mean number of meals per 24 h of 0.14. Cows ate a mean proportion of 0.59 of their meals during the day. Analysis of experimental data suggested that

pooling, conducted in previous studies, has probably led to a quantitative underestimation of the increase in starting probability with time since the last meal but not a qualitative misinterpretation of the direction of change in the starting probability.

Simulation studies showed that pooling had no serious consequences when the mean number of meals per 24 h, or the variation about this mean, was low. However, as the number of meals per 24 h and variation increased, pooling led to conclusions that may wholly misrepresent both magnitude and direction of the change in starting probabilities calculated separately for the individuals or for day and night. This may explain why the results of some published studies seem not to agree with biological principles of food intake regulation.

3.2. Introduction

Optimisation of both the quantity and quality of feeds that are supplied to animals requires understanding of how animals regulate food intake (Forbes, 1985). Analysis of short-term feeding behaviour can increase such understanding (Dürst *et al.*, 1993). Short-term feeding behaviour is frequently recorded in terms of feeding events such as pecks, bites or visits to the feeder (Slater, 1974; Orr *et al.*, 1997; Tolkamp and Kyriazakis, 1997). Such feeding events are generally clustered in time and can be grouped into bouts or meals using a meal criterion (Slater and Lester, 1982; Sibly *et al.*, 1990; Chapter 2). Previous work has shown that meals are a biologically relevant unit for the analysis of feeding behaviour (Tolkamp *et al.*, 2000). Only after identification of meals can parameters such as pre- and post-prandial correlation coefficients (Le Magnen and Devos, 1980) and the likelihood of animals starting a meal (Simpson and Ludlow, 1986) be calculated. Such variables are considered relevant to determine how variation in short-term feeding behaviour is related to variation in long-term voluntary intake and animal performance (Simpson and Ludlow, 1986; Tolkamp *et al.*, 2002).

To calculate parameters such as prandial correlations or starting probabilities, large data sets are generally required for reliable estimates. Collection of large individual data sets can be laborious, time consuming and expensive (Machlis *et al.*, 1985; Leger and Didrichsons, 1994). To minimise this, data of, for example, individual animals, collected during the day as well as during the night, are often pooled to provide data sets that are large enough for robust statistical analysis (Leger and

Didrichsons, 1994; Morgan *et al.*, 2000a; Tolkamp *et al.*, 2002). Previous analyses have shown, however, that pooling may influence the interpretation of data (Machlis *et al.*, 1985; Martin and Kraemer, 1987; Leger and Didrichsons, 1994; Morgan *et al.*, 2000b). Indeed, the effect that inappropriate pooling can have on the magnitude of prandial correlations, that is artificially increasing their significance, is already well documented for monogastrics (Panksepp, 1973; de Castro, 1975; Bigelow and Houpt, 1988; Demaria-Pesce and Nicolaidis, 1998).

Much less work has been done on the manner in which the probability of animals starting a meal changes with time since the last meal and how this is affected by pooling. Starting probability is relevant from at least two perspectives. In the first place, it directly determines the shape of the frequency distribution of intervals between meals. Meal criteria are generally estimated after fitting a model to the distribution of intervals between feeding bouts, including intervals between meals (Chapter 2). What is, or is not, an appropriate model to describe the distribution of intervals between meals is, therefore, directly dependent on the starting probability. Indeed, to decide between alternative probability density functions, analysis of starting probability may be more powerful than the goodness of fit of these models to the frequency distribution itself (Chapter 2). Knowledge of starting probability is, therefore, essential, even before feeding events can be properly grouped into meals.

Apart from this methodological issue, starting probability gives information on the manner in which short-term feeding behaviour is, or is not, ordered. The probability of starting a meal has, therefore, also direct biological implications because it gives

information about the mechanisms underlying short-term feeding behaviour. There are strong suggestions in the non-ruminant literature, however, that pooling of data can have a profound effect on the estimated starting probability (Morgan *et al.*, 2000b). Two potential types of pooling have been identified that may affect the starting probability and, therefore, the conclusions that may be drawn about the underlying mechanisms that regulate feeding behaviour. These types are pooling across day and night and pooling across individuals.

The objectives of this study were, therefore, twofold. The first was to estimate how conclusions from analyses of cow feeding behaviour may be affected by pooling. To achieve this, detailed feeding behaviour data was utilised to investigate the effects of pooling across day and night or across individuals on the estimated probability of cows starting a meal. In addition, this analysis provided parameter estimates for novel simulation models that were explored in the second part of the chapter. The objective of these simulation studies was to extend the analysis of the effects of pooling to conditions not encountered in these experiments. These include situations in which there is more variation between individuals in the number of daily meals that are consumed and a larger diurnal variation (as, for instance, can be observed in pigs; Morgan *et al.*, 2000b). Therefore, the aim was to determine under which conditions pooling across either day and night, or across individuals, could affect the starting probability and the interpretation of short-term feeding behaviour.

3.3. Materials and methods

3.3.1. Experimental conditions

Data were collected during two experiments, described by Tolkamp *et al,* (1998b) (experiment one; **Exp. 1**) and by Tolkamp *et al.* (1998c) (experiment two; **Exp. 2**). Materials and methods relevant to the present study are summarised below.

Sixteen (Exp. 1) and 18 (Exp. 2) Holstein-Friesian cows were used in experiments that took place at the Langhill Dairy Cattle Research Centre (Edinburgh, Scotland). Cows were kept in a yard (as described by Tolkamp and Kyriazakis, 1997) which was predominantly lit by natural light, with additional low-level light supplied during the night. Cows left the yard twice daily during milking, which lasted for up to 60 minutes (between 06:00 and 08:00 and between 16:00 and 17:30). The yard was equipped with computerised food dispensers (Insentec B.V., Marknesse, The Netherlands) as described by Tolkamp *et al.* (1998a). Each food dispenser recorded the time when individual cows entered and exited the feeders. Access to the feeders was continuous except during milking and between about 08:00 and 09:30 when food residues were removed from the bins and fresh mixed food was supplied. The foods were mixtures of 70% grass silage and 30% concentrate on a fresh weight basis. Approximately three-quarters of the daily food was offered in the morning, with the remaining food added to the bins during the afternoon milking. The quantities of foods offered were calculated daily to allow at least 10% refusals.

Water was available *ad libitum* from two troughs situated one near each end of the row of food dispensers. In both experiments cows had access to 12 feeders. A total of 79,386 and 13,675 visits to the feeders were recorded during Exp. 1 and Exp. 2, respectively.

3.3.2. Estimating meal criteria

Recorded feeding behaviour consisted of visits to feeders, which were clustered into meals using a meal criterion. A meal criterion is defined as the longest non-feeding interval between visits to a feeder that can be considered part of a meal. In Chapter 2 a model was developed that used two Gaussian distributions and a Weibull distribution to describe the $\log_e$-transformed lengths of two populations of within-meal and one population of between-meal intervals, respectively. The probability density function (**pdf**; Everitt, 1998) of this model is given in Appendix A. Meal criteria were estimated from model parameters as detailed in Chapter 2.

3.3.3. Calculating the probability of cows starting a meal

The probability of a cow starting a meal, in relation to time since the last meal, was calculated as the proportion of intervals with duration $> z$ minutes and $\leq z + 15$ minutes, divided by the proportion of intervals $> z$ minutes. Observed probabilities were calculated directly from the data. Predicted probabilities were calculated from the Weibull fit to the distribution of the population of intervals between meals, using

the estimated parameter values for the scale (α) and the shape (c) parameters in the Weibull cumulative distribution function (Johnson *et al.*, 1994), as explained in Chapter 2. Starting probabilities were calculated for each individual separately and for all data pooled. Median interval lengths between meals were plotted against time of day (according to the methodology of Morgan *et al.*, 2000b). Visual inspection of the graph showed that these lengths were shorter than average between approximately 08:00 until 20:00 and this was classified as day. A between meal interval was classified as a day interval if more than half of its length fell within this period and as a night interval otherwise. Starting probabilities were also calculated for the day and the night intervals separately. To avoid random fluctuations in the starting probability due to low numbers of observations, the starting probabilities were only calculated if the number of intervals longer than z minutes was greater than 100.

3.3.4. Simulation models

3.3.4.1. General

Simulation models were constructed to systematically investigate how diurnal variation and variation between individuals could influence the effects of pooling on the calculated probability of animals starting a meal. The simulation models were constructed using Fortran 90 (Ellis *et al.* 1994) and specific mathematical routines (see below) from the NAG library (NAG, 1993).

3.3.4.2. *Model parameterisation*

To construct and parameterise the simulation models it was necessary to make assumptions about distributions of, and relationships between, model parameters. The assumptions were made after examination of the literature and analysis of Exp. 1. Previous work has demonstrated that Weibull distributions are able to describe the between feeding intervals of individual cows (Chapter 2). Since the Weibull also gave an adequate description of the frequency distributions of day intervals and night intervals separately, the same model was used to describe the between meal intervals from day and the night separately, and from each individual separately. When a Weibull distribution is fitted to a population of between meal intervals, the c and α parameters are estimated such that the fit of the model is maximised. When the average number of meals per 24 h varies, this affects the mean interval length between meals. Therefore, at least one of the c or α parameters must necessarily vary to maximise the fit of the Weibull distribution. Regression analysis was used to quantify how the c and α parameters were related and how c and α varied in relation to the average number of meals per 24 h. Subsequently, an iterative bisection algorithm (Bruden and Faires, 1985) and NAG library routine D01AMF were used to calculate the relationship between the α parameter and the average number of meals per 24 h. To make the model stochastic, the variation between individuals was assumed to be normal and assessed using a Kolmogorov-Smirnov normality test (Minitab, 1998).

3.3.4.3. *Model testing*

Prior to using the assumptions and relationships (derived from Exp. 1) to parameterise the simulation models, these relationships were compared with those derived from an independent data set (Exp. 2). This ensured the relationships used to parameterise the models were robust. To this end, the observed starting probabilities were calculated for the between meal intervals from the day and the night and from individuals and compared to the starting probabilities estimated from the parameters of the fitted Weibull distributions. The relationships between Weibull parameters and the average number of meals per day were also examined to determine if they were similar to those calculated from Exp. 1. The suitability of a normal distribution to describe the individual average number of meals per 24 h for the cows in Exp. 2 was also assessed using Kolmogorov-Smirnov normality test (Minitab, 1998). Additionally, the average number of meals per 24 h and the proportion of meals that occurred during the day were calculated from Exp. 2. This information was used to simulate a number of observations that was approximately equal to the number of observations in Exp. 2 to estimate the effects of pooling across day and night. A good agreement between starting probabilities that were calculated from the observed feeding behaviour and those predicted by the model would demonstrate that the model assumptions were reasonable. Similarly, the population mean of the average number of meals per 24 h and the variation about that mean was calculated from Exp. 2. These data were used to simulate the effects of pooling across individuals with the model developed on the basis of Exp. 1. Therefore, models that were parameterised with the data from Exp.1 were tested against the data from Exp. 2.

3.3.4.4. *Pooling across day and night*

The average number of meals per 24 h and the proportion of these meals occurring during the day were varied systematically across a range of 6 to 18, and 0.5 to 0.8, respectively. These ranges encompass the values typically seen in the literature for large farm animals (e.g. de Haer and Merks, 1992; Musial *et al.,* 2000; Quiniou *et al.*, 2000a; Beauchemin *et al.*, 2002; Schwartzkopf-Genswein *et al.*, 2002; Tolkamp *et al.,* 2002). To obtain a good picture of the effects of pooling, a large number (200,000) of between meal intervals were simulated. For each combination of the average number of meals per 24 h and the proportion of meals occurring during the day this information was used to parameterise two Weibulls to generate two populations of intervals representing day and night. These populations were simulated using NAG library routine G05DPF. Starting probabilities were then estimated for the day and night populations separately and for all data pooled.

3.3.4.5. *Pooling across individuals*

The population mean of the average number of meals per 24 h and the proportional CV were varied systematically across a range of 6 to 18 and 0.0 to 0.3, respectively. This range in CV, of the average number of meals per 24 h, encompassed the typical values found in the literature for cattle (e.g. Beauchemin *et al.*, 2002; Schwartzkopf-Genswein *et al.*, 2002; Tolkamp *et al.,* 2002). Therefore, for a given population mean of the average number of meals per 24 h and a given CV the NAG library routine G05DDF selected an average number of meals per 24 h from the normal distribution,

for each simulated individual. The relationships between the average number of meals per 24 h and the Weibull parameters were then used to parameterise a Weibull distribution for an individual and its between meal intervals were simulated, for 100 days, using NAG library routine G05DPF. To obtain a clear picture of the effects of pooling across individuals, this was repeated for a very large number (1000) of simulated individuals. This produced a pooled data set of between approximately 600,000 and 1,800,000 simulated observations for 6 and 18 meals per 24 h, respectively. For each combination of the population mean of the average number of meals per 24 h and the CV, the simulated between meal intervals from all the individuals were pooled. Subsequently, the probability of animals starting a meal was calculated both from the simulated intervals and from the parameters of the Weibull distribution that was fitted to the pooled data.

3.4. Results

3.4.1. Observed variation in feeding patterns

Figure 3.1a gives the starting probability calculated after pooling the observations from the 16 cows in Exp. 1. There is a good agreement between the observed starting probability and that calculated from the parameters of the Weibull distribution fitted to the pooled data. The probability of cows starting a meal increased continuously, however the rate of increase in starting probability was faster at shorter, and slower at longer, interval lengths. This resulted in a 1.9 fold increase in starting probability between interval lengths of 2 and 6 h. The individually fitted Weibull distributions resulted in meal criterion estimates and average number of meals per 24 h as given in Table 3.1. Individual variation resulted in a CV of 0.14 about the population mean of the average number of meals per 24 h. Figure 3.1b shows that the probability of starting a meal, in relation to the time since the last meal, was higher during the day than during the night at any given interval length since the last meal. The fit of the starting probability calculated from the parameters of the Weibull distribution to the starting probability calculated from the day and from the night observations are good for the shorter interval lengths. However, for intervals longer than about 6 h the observed starting probabilities deviated systematically from the predictions. The increase in starting probability, between interval lengths of 2 and 6 h, was 2.1 fold during the day and 2.5 fold during the night. Therefore, the increase in starting

probability estimated from the pooled data (1.9 fold, Figure 3.1a) was lower than during either the day or the night (Figure 3.1b).

Table 3.1. *Experimental and meal characteristics*

	Experiment 1	Experiment 2
Number of cows	16	18
Days in experiment (mean ± s.e.)	156.5 ± 12.9	28 ± 0.0
Meal criteria (minutes ± s.e.)	25.6 ± 1.5	35.5 ± 3.3
Average number of meals per 24 h ± s.e.	6.01 ± 0.2	5.72 ± 0.3
Range of average number of meals per 24 h	4.7 – 7.4	3.8 – 7.6
Proportion of meals occurring in the day	0.59 ± 0.01	0.60 ± 0.01
Range of proportion of meals occurring in the day	0.54 – 0.64	0.51 – 0.70

Figure 3.1c shows the starting probability of two cows and the starting probability calculated from the individually fitted Weibull distributions. These cows were selected as they had the highest (cow 1 = 7.4) and lowest (cow 2 = 4.7) average number of meals per 24 h during Exp. 1. Such individual variation in feeding patterns clearly had a marked effect on the associated probability of cows starting a meal. The increase in starting probability was 2.1 and 2.4 fold for cows 1 and 2, respectively, between interval lengths of 2 and 6 h. Again, the increase in starting probability estimated from the pooled data (1.9 fold, Figure 3.1a) was lower than for either cow 1 or cow 2 (Figure 3.1c). The increase in the probability of cows starting a meal was adequately predicted by the parameters of the fitted Weibull models for

individual cows. It is evident from Figure 3.1c that the cow with the lowest number of meals (and, therefore, the lowest number of observations) tends to show most random variation around the observed trend.

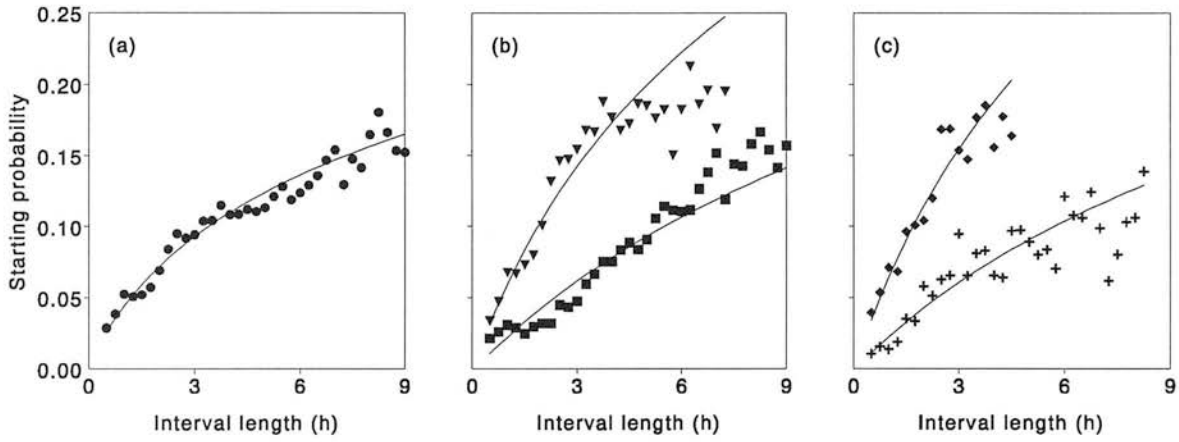

Figure 3.1. *The probability of cows, from Experiment 1, starting a meal in the next 15 minutes in relation to the length of the preceding non-feeding interval: (a) calculated from the pooled observations (dots). (b) calculated from the day (triangles) and the night (squares) observations. (c) calculated from the observations of cow 1 (diamonds) and cow 2 (crosses). Lines represent the starting probability calculated from the parameters of the fitted Weibull distributions.*

3.4.2. Simulation models

3.4.2.1. Model parameterisation

Figure 3.2a shows the relationship between the c and α parameters of the individually fitted Weibull distributions from Exp. 1. Because no significant correlation was found between these variables ($P > 0.05$) they were assumed to be independent. Figure 3.2b shows that the c parameter was not systematically affected by the average number of meals per 24 h ($P > 0.05$). Therefore, in the simulation models this parameter was assumed to be constant at the mean value of the c parameters (16.9). Figure 3.2c shows that the α parameters of the individually fitted Weibull distributions varied in a curvilinear fashion with the individuals' average number of meals per 24 h. For the cows in Exp. 1, the total between meal interval duration per 24 h (data not shown) averaged 20.6 h and was independent of the average number of meals per 24 h ($P > 0.05$). Consequently, the average between meal interval length was equal to 20.6 h divided by the average number of meals per 24 h. With the c parameter maintained at 16.9, the corresponding unique value for the α parameter could then be calculated for each average number of meals per 24 h. This was repeated across the range of average number of meals per 24 h used in the simulation studies. The result was a curvilinear relationship between the α parameter and the average number of meals per 24 h (Figure 3.2c) which was used in the subsequent simulation modelling. As the distribution of the individuals' average

number of meals per 24 h in Exp. 1 was not different ($P > 0.05$) from normal, the assumption of normality was used in the subsequent simulation modelling.

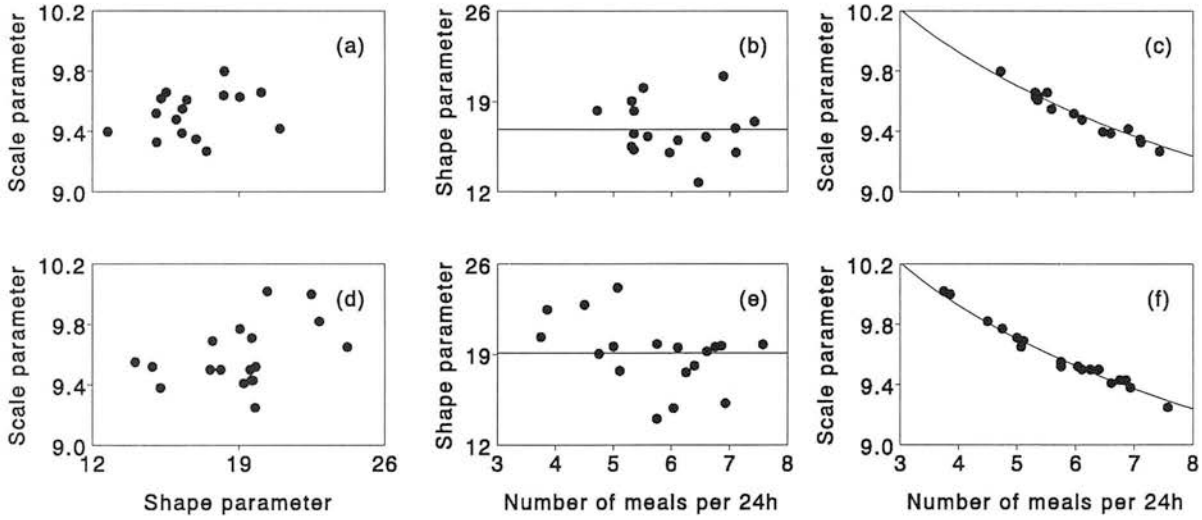

Figure 3.2. *The relationships between the average number of meals per 24h and the shape and scale parameters of individually fitted Weibull distributions: a and d give the relationships between the shape and scale parameters (dots). b and e give the relationships between the average number of meals per 24 h and the shape parameters (dots). The line represents the mean value of the shape parameters. c and f give the relationships between the average number of meals per 24 h and the scale parameters (dots). The line represents the calculated relationship (see main text) between the two variables. a, b and c relate to Experiment 1, whereas d, e and f relate to Experiment 2.*

3.4.2.2. *Model testing*

The suitability of using the relationships derived from Exp. 1 to parameterise the simulation models was assessed by comparison of these relationships to the analogous relationships calculated from Exp. 2. Figure 3.3b shows the calculated starting probability during the day and during the night and the predicted starting probabilities calculated from the parameters of the fitted Weibull distributions for Exp. 2. Figure 3.3c gives the starting probability of the cows, from Exp. 2, with the highest (cow 3 = 7.6) and lowest (cow 4 = 3.8) average number of meals per 24 h, and the predicted starting probability calculated from the parameters of the fitted Weibull distributions. In both cases the fitted Weibull distribution predicted similar starting probabilities to those observed. Figure 3.3 is based on less observations and shows greater scatter about the fitted Weibull than the equivalent graphs from Exp.1 (Figure 3.1). Figure 3.2d, 3.2e and 3.2f show the relationships between the average number of meals per 24 h and the parameters of the fitted Weibull distributions for the cows in Exp. 2. These Figures show that that the c and α parameters, and the c parameter and the average number of meals per 24 h, were not significantly ($P > 0.05$) correlated, in agreement with Exp. 1 (Figure 3.2a and 3.2b). Figure 3.2f demonstrates that the α parameters of the individually fitted Weibull distributions were significantly correlated ($P < 0.001$) with the individuals' average number of meals per 24 h and that the relationship was not different to that derived for Exp. 1. The distribution of the individuals' average number of meals per 24 h in Exp. 2 was not different ($P > 0.05$) from normal.

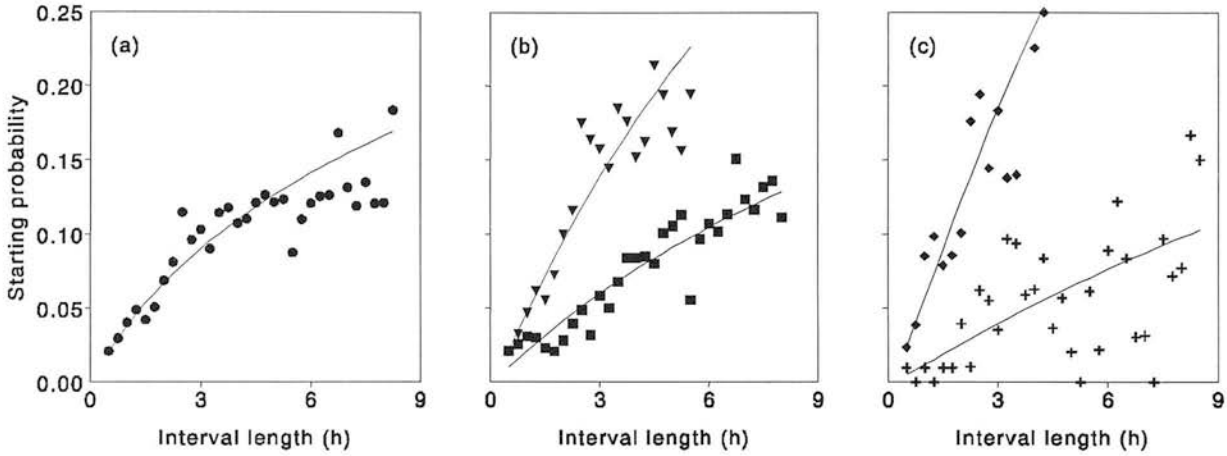

Figure 3.3. *The probability of cows, from Experiment 2, starting a meal in the next 15 minutes in relation to the length of the preceding non-feeding interval: (a) calculated from the pooled observations (dots). (b) calculated from the day (triangles) and the night (squares) observations. (c) calculated from the observations of cow 3 (diamonds) and cow 4 (crosses). Lines represent the starting probability calculated from the parameters of the fitted Weibull distributions.*

Figure 3.4 shows the results from the assessment of both simulation models. The starting probabilities calculated from the day intervals (Figure 3.4a) and from the night intervals (Figure 3.4b) in Exp. 2 are compared to predictions from the simulation models. Figure 3.4c gives the starting probabilities calculated from the pooled individuals in Exp. 2 and the predictions from the model simulating pooling across individuals. The observations and the simulations are found to coincide well across the range of interval lengths for both simulation models. Use of the mathematical relationships between the Weibull parameters and the average number

of meals per 24 h, derived from Exp. 1, for further simulation studies were therefore considered to be justified.

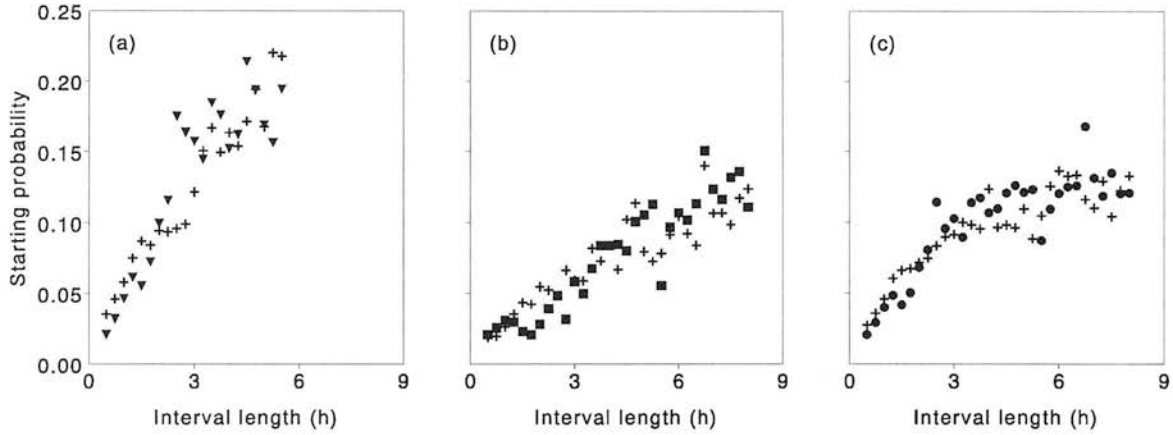

Figure 3.4. *The probability of cows starting a meal in the next 15 minutes in relation to the length of the preceding non-feeding interval: (a) calculated from the observations during the day from Experiment 2 (triangles) and predicted by the model simulating data from the day (crosses). (b) calculated from the observations during the night from Experiment 2 (squares) and predicted by the model simulating data from the night (crosses). (c) calculated from the observations pooled across individuals from Experiment 2 (dots) and predicted by the model simulating pooling across individuals (crosses).*

3.4.2.3. Pooling across day and night

Figure 3.5 summarises the results from the simulation of pooling across day and night (the results from simulation of 18 meals per 24 h and the simulations with a

proportion of 0.8 meals occurring during the day are omitted as the results follow the trends discussed below). When there is no diurnal variation (Figure 3.5a and 3.5d), that is when the proportion of meals consumed during the day and during the night is the same, the starting probabilities from the day, night and pooled data are necessarily very similar. Figure 3.5b shows that when a proportion of 0.6 of 6 meals per 24 h occur during the day, the pooled starting probabilities approach the simulated day probabilities at shorter interval lengths. However, at longer interval lengths the pooled probabilities are more similar to the simulated night probabilities. This results in the starting probability of the pooled data continuously increasing but at a slower rate at longer interval lengths than either day or night probabilities. Therefore, the pooled results are in agreement with the increase in starting probabilities observed during the day and the night, although this increase is quantitatively underestimated. The effect of pooling is more extreme when a proportion of 0.7 of the meals occur during the day (Figure 3.5c). The pooled starting probability then decreases for intervals greater that 3h, i.e. the results are in agreement with neither the day nor the night data. When the average number of meals per 24 h increases further, a similar but more extreme pattern is observed. This is demonstrated by comparison of Figure 3.5c (6 meals per 24 h) and Figure 3.5f (12 meals per 24 h) which give the simulated results when a proportion of 0.7 of meals occur during the day. When the number of meals per day increases the pooled starting probability is seen to approach the night probabilities at shorter interval lengths.

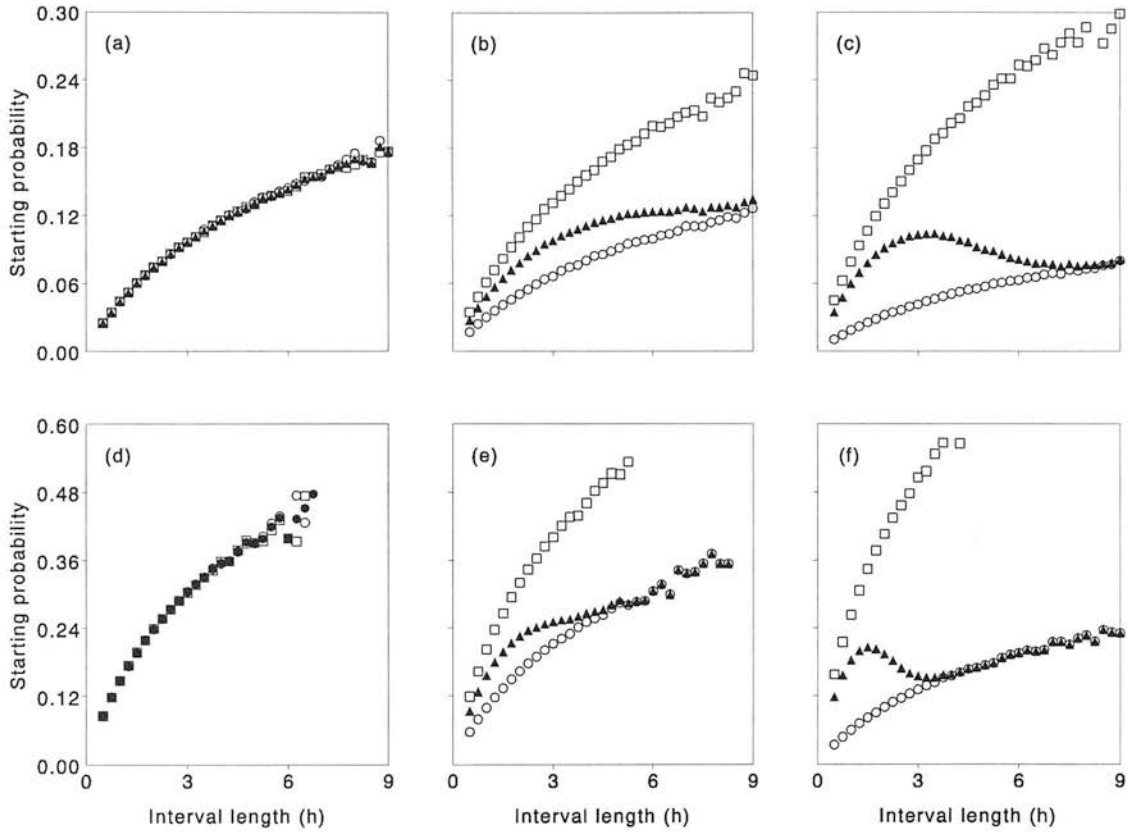

Figure 3.5. *The probability of animals starting a meal in the next 15 minutes in relation to the length of the preceding non-feeding interval, as calculated from the model simulating day (open squares), night (open circles) and pooled day and night (triangles). Simulated for the given average number of meals per 24 h (and proportion of meals occurring in the day) of: (a) 6 (0.5), (b) 6 (0.6), (c) 6 (0.7), (d) 12 (0.5), (e) 12 (0.6) and (f) 12 (0.7).*

3.4.2.4. Pooling across individuals

Figure 3.6 gives a graphical summary of the output from the model simulating pooling across individuals which vary stochastically in their average number of

meals per 24 h. The results from simulating 18 meals per 24 h and for CV of 0.3 are omitted as the results follow the trends discussed below. When there is no variation in average number of meals per 24 h between simulated individuals, i.e. CV of 0.0, then there is a smooth increase in starting probability which necessarily coincides well with starting probabilities calculated from the parameters of the Weibull distribution (Figure 3.6a and 3.6d). When the variation between simulated individuals increases then the effect of pooling across individuals becomes more evident. With an average of six meals per day and CV of 0.1 (Figure 3.6b) the starting probability continues to increase over the whole range of interval lengths. However, the rate of increase in starting probability calculated from the fitted Weibull distribution is slightly overestimated at long interval lengths. When the CV is 0.2, for 6 meals per 24 h, then the pooled starting probability is seen to be approximately constant after 6 hours. Figures 3.6e and 3.6f show that when the population of simulated individuals has on average 12 meals per 24 h then a similar, but more extreme, pattern emerges. With a CV of 0.1 (Figure 3.6e) the deviation from the Weibull predictions and the levelling off of the starting probability begin at shorter interval lengths, compared to the simulation of 6 meals per 24 h (Figure 3.6b). When there is both considerable variation in the number of meals per 24 h (CV of 0.2) and many meals per 24 h (e.g., 12) pooling of individuals results in, first an increasing, and subsequently a decreasing, starting probability (Figure 3.6f). In such cases, a fitted Weibull gives a poor description of the frequency distribution of intervals between meals and, consequently, poor predictions of the change in starting probability at longer interval lengths.

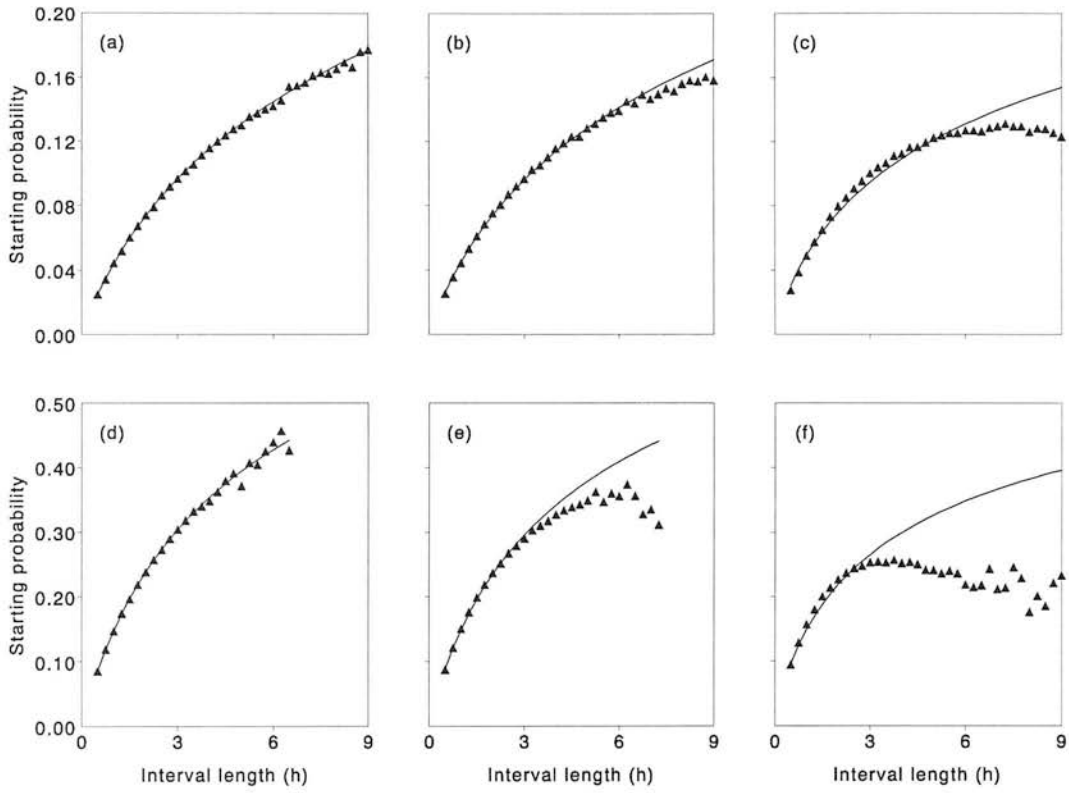

Figure 3.6. *The probability of animals starting a meal in the next 15 minutes in relation to the length of the preceding non-feeding interval, as calculated from the model simulating pooling across individuals (triangles). Simulated for the given population mean in the average number of meals per 24 h (and proportional CV about that mean) of: (a) 6 (0.0), (b) 6 (0.1), (c) 6 (0.2), (d) 12 (0.0), (e) 12 (0.1) and (f) 12 (0.2). Lines represent the starting probability calculated from the parameters of the fitted Weibull distributions.*

3.5. Discussion

3.5.1. General

The analysis of short-term feeding behaviour may give insights into how the motivation to eat changes with time. This could lead to a better understanding of how animals control their food intake (Simpson and Ludlow, 1986). This motivation is thought to be related to satiety, which has been defined by Le Magnen (1985) as "a passive state of no hunger. The termination of a meal, ... is the onset of satiety". It is generally assumed that as satiety decreases with time since the last meal, there will be a continuously increasing probability of the next meal starting (see Tolkamp *et al.*, 1998a, for further discussion). Therefore, the analysis of starting probabilities is a biologically meaningful way of studying feeding behaviour and of testing whether appropriate models have been used for its analysis.

Before feeding behaviour can be analysed, decisions have to be made about how to organise the available data. One such decision is whether to pool data, thereby increasing the quantity of data available per analysis. This can be advantageous, as was demonstrated within the present study by comparison of individually calculated starting probabilities, and those calculated from data pooled across individuals. Cows with relatively low starting probabilities will eat fewer meals and, consequently, have fewer between meal intervals per day. Such cows tend to show more random variation around the general trend than cows with higher starting probabilities (see

Figures 3.1c and 3.3c). Random variation in starting probabilities can be reduced by pooling of data across animals (see Figures 3.1a and 3.3a). Therefore, it may seem attractive to pool data in order to clarify the patterns of observed behaviour.

Data pooling has, however, been the subject of considerable debate over recent years. Following the paper of Machlis *et al.* (1985) the consequences of pooling data have been further investigated by Martin and Kraemer (1987) and Leger and Didrichsons (1994) using observed data, and by Arsenos *et al.* (2000) and Pomar *et al.* (2002) using simulation studies. These studies have demonstrated that the results from analysis of pooled data do not always represent the patterns of behaviour of the individuals that contributed to the pooled data. Within the field of feeding behaviour, it has been suggested that inappropriate pooling across day and night (Morgan *et al.*, 2000b) or across individuals (Tolkamp *et al.*, 2000) could have significant consequences for the interpretation of starting probabilities. Therefore, an understanding of the effects of data pooling is deemed integral to a better understanding of short-term feeding behaviour.

It is evident that the effects of pooling of data obtained from different individuals or from different periods will depend on the variation between individuals or between periods. Therefore, conclusions with regard to the effects of pooling based on the observations obtained in any actual experiment are limited to the variation encountered in that experiment. To be able to draw more general conclusions about the effects of pooling, data sets can be simulated that include a wider range of variation. Before simulation can yield any results, the parameters for the simulation

models have to be derived. To that end, an existing experiment was analysed to derive parameter values. The appropriateness of these parameters was subsequently tested with data obtained in another experiment. These parameters were then used to simulate data sets with a range of variation between individuals and between periods to estimate effects of pooling under such conditions.

3.5.2. Experimental data

Analysis of Exp. 1 shows that cows ate proportionally 0.59 of their meals during the day and this was associated with a higher probability of cows starting a meal during the day. This analysis (Figure 3.1b) also suggested that the observed starting probabilities calculated from the day and the night intervals merge beyond interval lengths of 7.5 h. This is related to the way intervals were classified into day or night. With this definition of day and night, a 10 h interval ending in the night was classified as a night or a day interval if 5.1 or 4.9 h, respectively, fell in the night period. However, it may be expected that such intervals would have similar starting probabilities, irrespective of whether they are classified as day or as night intervals. Short intervals are less likely than long intervals to start in the day and end in the night (or vice versa). Although such 'mixed' intervals are only a small proportion of all intervals, they occur relatively frequently for longer intervals. This explains why starting probabilities are clearly separated at shorter, but tend to merge at longer interval lengths. Nevertheless, analysis shows that the starting probability calculated from the data pooled across day and night was largely determined by the day probabilities at short intervals and the night probabilities at longer intervals.

Therefore, pooling of data across day and night led to some underestimation of the rate of change in starting probability with time since the last meal. Despite this, the pooled starting probabilities continued to increase with time since the last meal. This is in agreement with both the trends observed during the day and the night and the expectations based on the satiety concept. It is also consistent with the results of Morgan *et al.* (2000b).

There may be several reasons for the lower starting probability at a given time after a meal that is observed during the night compared to the day. Theoretically, meal size could have a considerable effect on subsequent starting probabilities. If cows consumed larger meals at night, this could have resulted in decreased probabilities at night after a given interval length. Two things would then be expected: (i) larger average meal sizes during the night than during the day and (ii) considerable post-prandial correlation coefficients. In fact, there is no evidence for either of these expectations. Work with cows on similar diets in the same facility showed that meal sizes tended to be smaller, not larger, during the night than during the day (Tolkamp *et al.*, 2002). The same was true for the data sets analysed in this paper. In addition, post-prandial correlations, although sometimes significant, were always very low in data sets obtained with cows under similar conditions, even when these are calculated only for a given period of the day (Tolkamp *et al.*, 2002, Chapter 5). It seems, therefore, highly improbable that an explanation can be found in this direction.

It seems likely that the probability of cows starting a meal is related, directly or indirectly, to day light. There are clear diurnal patterns, affected by day length, in the circulating levels of hormones related to food intake regulation such as melatonin (e.g. Redondo *et al*, 2003). It seems probable that such internal signals, which are known to be related to food intake have an effect on the hunger/satiety mechanisms and, as a result, on the diurnal rhythm of the probability of animals starting a meal. In addition, there may be external stimuli affecting short-term feeding behaviour that are indirectly related to day length. For instance, the provision of fresh food and milking occur largely during the day and these may be such stimuli. It is generally thought that such external and internal signals interact and determine feeding behaviour (Berthoud, 2002) and this may result in the diurnal pattern that was observed.

Individual animals may also differ in their feeding patterns (Slater, 1974) and consequently display differing starting probabilities (Figure 3.1c). The work within this chapter demonstrates that pooling of feeding behaviour data across individuals results in pooled starting probabilities that increase at a slower rate than when individuals are assessed separately (Figure 3.1). This is the result of the cows with many meals per 24 h having many short intervals between meals and, consequently, contributing many of the data used to calculate starting probabilities at short interval lengths. Conversely, cows with few meals per 24 h contribute very few short, but many longer, intervals. Therefore, pooling of data from such cows leads to a pooled starting probability that is largely determined, at short intervals, by the probabilities of cows with many meals and at long intervals by the probabilities of cows with few

meals per 24 h. Under the conditions of this study, pooling of such animals leads to a rather slow increase in starting probability, especially at longer interval lengths. However, pooling did not lead to changes in starting probability with time since the last meal that were in contrast with the satiety concept. This was related to the limited diurnal variation observed in this data set.

3.5.3. Simulation models

The second part of the analyses undertaken in this study concentrated on the development, testing and use of simulation models. These models were developed to systematically investigate how pooling of behavioural data from animals which display more diurnal variation or vary more strongly in their average number of meals per 24 h than these experimental animals could influence the calculated starting probability. Such an exercise is useful because it allows identification of conditions under which pooling of data across animals or across day and night may lead to unacceptable conclusions.

Construction of these models required the use of information from the literature and analysis of experimental data, to derive model parameters. In the experiments reported in this chapter, the fitted Weibull distributions predicted starting probabilities that were similar to those observed during the day and during the night and from individuals, which is in agreement with Tolkamp *et al.* (2002) and Chapter 2. The relationships between the parameters of the Weibull distribution and the average number of meals per 24 h were remarkably similar in both experiments. The

mean value of the *c* parameter was found to be higher in Exp. 2 compared to Exp. 1, however the simulation model was not sensitive to small changes in the value of this parameter. No evidence was found in either experiment that the individual variation in average number of meals per 24 h differed from a normal distribution, similar to previous findings with cows (Tolkamp *et al.*, 2000) and with pigs (Allen, 2000). Analysis of the fully constructed and parameterised models showed that both accurately simulated the observed starting probabilities, of an independent data set (Figure 3.4). It is therefore considered that the assumptions made within these models, and their subsequent use in the simulation studies, was justified.

The ranges over which the model variables were changed, i.e. proportion of meals occurring in the day, the average number of meals per 24 h and CV about this mean as a result of individual variation, were selected to represent the ranges typically seen in such studies (e.g. Metz, 1975; Tolkamp *et al.*, 2002). Simulation modelling demonstrated (Figures 3.5 and 3.6) that the effect of pooling became more apparent when the number of meals per 24 h increased. For a given mean number of meals per 24 h, the effects of pooling became stronger with increases in variation between individuals and with more pronounced diurnal meal patterns (Figures 3.5 and 3.6). When a high level of variation between individuals was combined with a large number of meals per 24 h, the pooled starting probability actually decreased (Figure 3.6). This occurred in spite of the fact that starting probabilities actually increased for each individual that contributed to the pooled data. Similarly, even when the starting probability increased during the day as well as during the night, the pooled starting probabilities could remain relatively constant (Figure 3.5), as was observed by

Morgan *et al.* (2000b) for pigs. Therefore, the results of this simulation work show that with sufficient variation between individuals or between day and night, the pooled starting probabilities can appear to be independent of the time since the last meal. A constant starting probability would suggest that meals occur randomly in time and this, in turn, will lead to the use of inappropriate models, such as log-frequency or log-survivorship curves, to identify meals. It is evident that the use of such models will lead to biologically inaccurate conclusions with regard to animals' short-term feeding behaviour, which disagree with the predictions of the satiety concept.

3.5.4. Wider implications

Data pooling, both across day and night and across individuals, is prevalent in the literature, including many studies of feeding behaviour (e.g. Le Magnen and Devos, 1980; Savory, 1981; de Haer and Merks, 1992). The finding, that inappropriate pooling could lead to biologically unsound conclusions, led to questions about whether pooling had influenced the interpretation of previous studies of feeding behaviour across a range of species. Two main reasons to be concerned about the effects of pooling are suggested by the findings of this study. The first is related to the methodological issue of clustering feeding events into meals. The concept of satiety and the way it is thought to affect short-term feeding behaviour has been around for a long time (e.g. Metz, 1975). Nevertheless, models contradicting such ideas, for instance negative exponentials, are still used to estimate meal criteria to this day (e.g. Stamer *et al.*, 1997; Quiniou *et al.* 2000b). Indeed, Quiniou *et al.*

(2000b) fitted such a model to the intervals between feeding events for individual pigs, pooled across day and night and determined that these pigs had approximately six meals per 24 h with a proportion of 0.78 of these occurring during the day. Use of such a model, however, implies that each individual's between meal intervals, when pooled across day and night, did not comply with an increasing starting probability (Tolkamp and Kyriazakis, 1999a). However, the simulation studies have shown one of the reasons why such models may still be considered appropriate by those who apply them. If data are very variable and if pooled data sets are used, the inappropriateness of such models may be hidden as a result of pooling. Pooling may then give the impression that animals start meals randomly and this can subsequently lead to inappropriate models to estimate meal criteria. Tolkamp and Kyriazakis (1999a) showed that such models frequently underestimate meal criteria. This will result in meals being erroneously split into two or more bouts that are then considered as meals *per se*. It is evident that this will affect all subsequent analyses and it should therefore be avoided.

The calculation and expression of how the observed and predicted starting probabilities change with time is therefore a useful way of exploring data. If the observed probabilities do not change as expected, i.e. follow the predictions of the satiety concept, an examination of the raw data may prove beneficial. Such analysis may disclose inappropriate pooling or failure to disaggregate two populations of data (Morgan *et al.*, 2000b). Further to this, when statistical models, e.g. Slater and Lester (1982); Tolkamp and Kyriazakis (1999a); Chapter 2, are used to estimate meal criteria, this allows a comparison of the starting probabilities predicted by these

models and those observed. Such a comparison will immediately reveal any discrepancies between the assumptions underlying the model and the observed feeding behaviour (e.g. Chapter 2). This may lead to improved models and, consequently, better understanding of food intake regulation.

3.5.5. Conclusions

The conclusions from this study are three-fold. Firstly, the analyses show that the probability of cows starting a meal is not greatly affected by pooling as long as the variation in feeding behaviour between individuals or between day and night is not very large. Therefore, in the previous analyses presented in Chapter 2 the effect, where data were pooled, is likely to have been quantitative underestimation of the increase in the starting probability with time, but not a qualitative misinterpretation of the direction of change. Secondly, the simulation models demonstrated that the conclusions from analysis of short-term feeding behaviour might be strongly affected by pooling. The probability of animals starting a meal, calculated from pooled data, may wholly misrepresent the individuals, or the day and night when assessed separately, if the individual or diurnal variation in the average number of meals per 24 h is sufficiently high. Analysis of the literature suggests that this phenomenon could have occurred in other studies of feeding behaviour in which data were inappropriately pooled. Lastly, it is strongly recommended that authors calculate and present the predicted and observed changes in the probability of animals starting a meal with time and compare this with expectations based on the satiety concept. This is an excellent way of investigating whether appropriate models were used to

describe feeding behaviour and whether or not inappropriate pooling has occurred. Such information would benefit the interpretation of analyses and, consequently, allow stronger conclusions.

CHAPTER FOUR

The relationship between meal composition and long-term diet choice*

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4.1. Abstract

When animals are offered a choice of foods, which are nutritionally complementary, they are able to select a consistent combination of these foods over long periods of time. Analysis of how such consistent diet choice is achieved, in terms of short-term feeding behaviour, may further our knowledge of how animals regulate nutrient intake. Previous work, on meal pattern analysis and on nutrient synchronisation, led to the hypothesis that animals may select a consistent diet within a meal. In three experiments cows were offered a choice between high (**H**) and low (**L**) protein foods and short-term feeding behaviour data were collected using computerised feeders. Feeding behaviour was first analysed in terms of visit characteristics. A greater average daily intake of H, relative to L, was more closely related to the ratio of H visits to L visits, than to differences in the intake per visit to feeders supplying H or L. Individual meal criteria were estimated, using a mixed distribution model, and visits were clustered into meals. Cows typically had around six meals per day. The observed frequency distribution of meal composition, in terms of the proportion of visits to H feeders, was determined. Subsequently, the observed visits were randomly re-clustered into bouts, consisting of the same number of visits as were observed in meals, and the frequency distribution of random bout composition was calculated. If the frequency distributions of meals and of random bouts coincide then this is evidence that cows do not regulate diet choice within a meal. Comparison of the frequency distributions of meals and random bouts provided no evidence that cows attempted to achieve their long-term average diet composition within a meal. Whether cows tried to achieve a consistent diet choice within a meal, by adjusting

their intake per visit depending on the food type visited and the proportion of visits to H feeders in a meal, was also investigated. There was no evidence that this occurred. In conclusion, these analyses have shown that cows did not attempt to select within a meal a consistent diet in terms of protein to energy ratio. Indeed, these data and the literature suggest that the timeframe over which the intake of energy and protein is regulated must be greater than a meal in a number of animal species.

4.2. Introduction

When offered a choice of foods, animals frequently select a consistent long-term diet (Larue-Achagiotis *et al.*, 1992; Forbes and Kyriazakis, 1995). For instance, animals with access to two similar foods that differ in protein content can select a consistent diet that meets their protein requirements (Kyriazakis *et al.*, 1990; Forbes and Shariatmadari, 1996). Consistent long-term diet selection is the result of shorter term feeding behaviour (Gill and Romney, 1994), and diet choice may be regulated in the short-term (Shariatmadari and Forbes, 1992). This would enable nutrient supply (for instance energy and protein) to be synchronised. This is thought to be important, especially for ruminant animals (Sinclair *et al.*, 1995; Kim *et al.*, 1999a; Witt *et al.*, 1999a). Therefore, analysis of short-term feeding behaviour may further our knowledge of the mechanisms that result in consistent long-term diet choice (Dürst *et al.*, 1993; Forbes, 1985).

The shortest unit of feeding that can be measured is often a visit to a feeder supplying one food type only (e.g., Barrio *et al.*, 2000; Bornett *et al.*, 2000). Therefore, diet choice cannot be expressed during a single visit. However, visits are usually clustered into meals (Morgan *et al.*, 2000b; Chapter 2) and meals are, therefore, the shortest biologically relevant unit in which diet selection can be expressed. The aim of this study is to investigate whether long-term average diet choice is a direct result of cows selecting a consistent diet within meals. A number of hypotheses were developed (see below) about the relationship between long-term average diet choice and diet selection within meals. Large data sets of dairy cow

feeding behaviour were used for severe statistical tests of these hypotheses. Analyses initially investigated feeding behaviour in terms of visits, then utilised a novel technique for the analysis of how meals are composed of visits. The results are discussed in relation to short-term feeding behaviour in other species.

4.3. Materials and Methods

4.3.1. General

The study utilises data collected during three diet choice experiments, described by Tolkamp *et al.* (1998c) (**Exp. 1**), Tolkamp *et al.* (1998b) (**Exp. 2**) and Tolkamp and Kyriazakis (1997) (**Exp. 3**). First, relevant materials and methods common to all three experiments are summarised. Subsequently, materials and methods relating to the individual experiments and their analyses are presented.

4.3.2. *Housing and daily routine*

The experiments took place at the Langhill Dairy Cattle Research Centre (Edinburgh, Scotland). Holstein-Friesian cows were kept in a yard, as described by Tolkamp and Kyriazakis (1997), for the duration of the experiments. They left the yard twice daily during milking, which lasted for up to 60 minutes (between 0600-0800 and between 1600-1730). During each of these periods cows received 0.5kg of parlour concentrates. On rare occasions cows also left the yard during short periods for management reasons such as foot trimming and pregnancy diagnosis. During these times cows had no access to the feeders.

4.3.3. Foods, food dispensers, and feeding regime

The yard was equipped with 28 computerised food dispensers (Insentec B.V., Marknesse, The Netherlands) as described by Tolkamp *et al.* (1998a). Each food dispenser consisted of a bin with a capacity of 160L, mounted on two load cells. Access to the bin was via a pneumatically operated gate, controlled by a computer. The gate was equipped with an antenna that responded to the transponder that each cow wore around her neck. Therefore, each cow could be given access to specific feeders. On entry to a feed bin, the cow's identification number, the weight of the bin and the time was recorded. When the cow left the feeder, the gate closed and the time was again recorded. The gate remained shut for at least ten seconds to allow stabilisation of the food bin prior to re-weighing. Time was measured to the nearest second, and weight to the nearest 0.1kg. Feeders were equipped with yokes during Exp. 1 and Exp. 2 to stop 'stealing' of food by non-experimental cows housed in the same yard.

Access to the feeders was continuous except during milking and between about 0800 and 0930 when food residues were removed from the bins and fresh mixed food was supplied. Approximately three-quarters of the daily food was offered in the morning, with the remaining food added to the bins during the afternoon milking. The quantities of foods offered were calculated daily to allow at least 10% refusals. Water was available *ad libitum* from two troughs situated one near each end of the row of food dispensers.

4.3.4. Experiment one

Foods were a mixture of 70% grass silage and 30% concentrate on a fresh weight basis and were formulated to have similar nutritional properties except for their protein contents, which were either high (**H**) or low (**L**). Table 4.1 shows that although effective rumen degradable protein (**eRDP**, defined by AFRC (1993) as slowly degradable protein plus 80% of quickly degradable protein) were similar, differences in the concentrate components of the foods resulted in two foods that differed in metabolizable protein yield (**MP**, defined by AFRC (1993) as an estimate of the availability of amino acids to the cow).

Feeding behaviour data were collected during four weeks from 18 cows. All cows had exclusive access to 12 feeders and 13,675 visits were recorded. Feeders 13 to 18 supplied L during the entire experiment. For the first two-week period feeders 7 to 12 supplied H and during the second two-weeks supplied a 50:50 ratio of H and L. The diet dilution in the second period of the experiment did not affect the proportion of L consumed (which remained 0.5) from feeders 13 to 18 (Tolkamp *et al.*, 1998c). This confirmed that these cows ate a diet at random. Therefore, for the purposes of the analyses presented below, the foods offered, across the two periods of Exp. 1, will be referred to as **H1** and **L1**. Because cows ate a diet that consisted of approximately 50% of each food the resultant cow pressure was equal for feeders supplying H1 and for those supplying L1 (Table 4.1). Cow pressure was defined as the number of cows that on average would attempt to eat at each feeder if all cows tried to eat at the same time.

Table 4.1. *Experimental structure and the foods offered.*

	Experiment 1	Experiment 2	Experiment 3
Number of cows	18	16	24
Days in experiment (mean ± s.e.)	28 ± 0.00	156.5 ± 12.90	14 ± 0.00
Food code	H1 L1	H2 L2	H3 L3
Mean cow pressure per feeder	1.50 1.50	1.86 1.85	1.68 0.89
Foods[a]			
DM	456 458	465 464	406 404
CP	179 167	185 131	197 149
MP	109 85	114 76	109 82
eRDP	106 108	119 87	131 100
ME	12.1 12.0	12.3 11.8	11.7 11.6
FME	10.1 10.0	10.4 10.1	10.0 9.9
NDF	327 330	344 332	383 401

[a]Dry matter (DM) (g/kg), crude protein (CP) (g/kg DM) and neutral detergent fibre (NDF) (g/kg DM) were determined directly from chemical analysis of the foods (see Tolkamp and Kyriazakis, 1997). Metabolisable protein (MP) (g/kg DM), effective rumen degradable protein (eRDP) (g/kg DM), metabolisable energy (ME) (MJ/kg DM), and fermentable metabolisable energy (FME) (MJ/kg DM) were determined from chemical analysis and tabulated values for ingredients used (AFRC, 1993).

4.3.5. Experiment two

Foods were a mixture of 70% grass silage and 30% concentrate on a fresh weight basis and were formulated to have similar nutritional properties except for their

protein contents, which were either high (**H2**) or low (**L2**). Table 4.1 shows that differences in the concentrate components of the foods resulted in two foods that differed in eRDP and MP yield.

Feeding behaviour data were collected, during 33 weeks from 16 cows. All cows had access to 12 feeders and 79,386 visits were recorded. Feeders 7 to 12 contained H2 and could be accessed by the experimental cows only. Feeders 13 to 18 contained L2 and could be accessed by experimental cows and by some non-experimental cows. Cows were recorded to select a diet that consisted of approximately 70% of H2. The access of non-experimental cows to additional feeders was manipulated such that the cow pressure per experimental feeder (Table 4.1) was kept approximately equal for both food types, despite the consistent non-random diet choice made by experimental cows (Tolkamp *et al.*, 1998b).

4.3.6. Experiment three

The foods were a mixture of 80% grass silage and 20% concentrate on a fresh weight basis and were formulated to have similar nutritional properties except for their protein contents, which were either high (**H3**) or low (**L3**). Table 4.1 shows that differences in the concentrate components of the foods resulted in two foods that differed in eRDP and MP yield.

Feeding behaviour data were collected from 24 cows, during weeks four and five of the experiment described by Tolkamp *et al.* (1998b). All cows had access to 28 food

dispensers. Feeders 8 to 14 and 22 to 28 contained H3. Feeders 1 to 7 and 15 to 21 contained L3. In addition, two control groups of six cows each had access to feeders supplying H3 and L3, respectively. A total of 20,971 visits to the feeders by choice cows were analysed. Cows were recorded to select a diet that consisted of approximately 70% of H3. This led to a cow pressure per experimental feeder (Table 4.1) that was approximately twice as high at feeders supplying H3 compared to those supplying L3.

4.3.7. Visit-based analyses

The visit was the shortest unit in which intake was recorded. Therefore, the feeding behaviour of individual animals in each experiment was first analysed in terms of visits. It was reasoned that if animals were eating randomly from the two foods (as concluded from earlier analysis of daily intakes during Exp. 1) then they would be expected to visit feeders supplying H (H feeders) as frequently as those supplying L (L feeders) and to eat an amount per visit that was independent of the food type consumed (hypothesis 1). Rejection of this hypothesis, for Exp. 1, would cast serious doubts on earlier conclusions (Tolkamp *et al.*, 1998c) that animals in this experiment ate at random. However, animals with a long-term diet choice that was different from random (e.g. Exp. 2 and Exp. 3) could achieve this through visiting feeders supplying a given food more frequently, or by consuming more than the average amount when visiting feeders supplying a given food, or some combination of these. As far as I know, an analysis of the short-term feeding behaviour of choice fed animals has never been published. However, Chambers *et al.* (1995) offered

locusts a diet choice and monitored their feeding behaviour. In that study, diet choice was achieved mainly by more frequent visits to one food type. This finding has been taken as the basis of the second hypothesis; that animals would achieve a non-random diet choice mainly by visiting feeders supplying a given food type more frequently, rather than regulating their intake per visit depending on the food type visited (hypothesis 2).

To facilitate comparisons between experiments, the following visit characteristics were calculated for each experiment; the intake, number of visits and visit duration at H feeders as a proportion of the total intake, total number of visits and total visit duration, respectively. If animals ate at random, then visit characteristics are expected to be unrelated to the type of food being consumed. Therefore, the calculated proportion of each of the above visit characteristics would be 0.5. Proportions were calculated from the average individual cow visit characteristics and t-tests used to determine if the calculated proportions differed significantly from 0.5.

The following visit characteristics were calculated to allow comparisons between food types within experiments; the mean intake per visit, mean duration per visit and mean intake rate during visits to feeders supplying H and L. This was achieved by fitting the following model (using average intake per visit as an example); $Y_{ij} = \mu + C_i + F_j + e_{ij}$, where Y_{ij} = average intake per visit for cow i when eating at feeder j, μ = average intake per visit, C_i = effect of cow i, F_j = effect of feeder j, e_{ij} = error term. One way ANOVA of the mean intake per visit for each feeder, with cow effect

accounted for, was used to test for an affect of the feed type that each feeder supplied on the mean intake per visit.

4.3.8. Meal-based analyses

A meal criterion is an estimate of the longest non-feeding interval, between visits to a feeder, which can be considered part of a meal. Calculation of a meal criterion allows the clustering of recorded visits into meals. The lengths of intervals, between visits to the feeders, were calculated for each cow as the time intervals between the end of one visit and the start of the next. All $\log_e$-transformed interval lengths between visits to the feeder that are greater than zero were utilised to estimate individual meal criterion using the method given in Chapter 2. After calculation of individual meal criteria, visits were clustered into meals for each cow. This resulted in meals that were composed of between one and more than ten visits. For each observed meal, the proportion of visits to H feeders was calculated. For instance, the proportion of visits to H feeders in a two-visit meal could be 0, 0.5 or 1 and in a three-visit meal 0, 0.33, 0.67 or 1.

In order to utilise the methodology of analysis described below it was essential to have the largest possible number of meals; i.e. pooling of information from individuals was required. Therefore it was first investigated if there was any evidence to suggest that the meal data from the individual cows should not be pooled. To that end, the individual SD's of the proportion of visits to the H feeder in a meal, were determined. Using a Kolmogorov-Smirnov normality test (Zar, 1996) the frequency

distribution of the coefficient of variation was tested to determine if it differed from a normal distribution. This was not the case in any of the experiments ($P > 0.15$). Therefore, the meal data were pooled within experiments to give a frequency distribution of meal composition.

Subsequently, it was tested whether the observed frequency distribution of meal compositions differed from a hypothetical frequency distribution of bout compositions, predicted using probability theory as described by Zar (1996). For each experiment, a frequency distribution of bout compositions was calculated with the assumption that each bout would consist of visits that were drawn randomly from the observed population of visits in that experiment. Therefore, the probability of visiting a feeder was assumed to be related only to the food type it supplied, with all feeders that supplied the same food type being equally likely to be visited. For instance, consider an experiment in which the proportion of visits to H feeders is 0.5. Then the probability that a randomly drawn visit from that population would be a visit to an H feeder would be equal to the probability that the visit would be to an L feeder, i.e. 0.5. Thus, the probability that a bout consisting of two randomly drawn visits would contain both visits to the same food type would be $0.5 \times 0.5 = 0.25$. Consequently, the probability that a bout would consist of one visit to each of the food types would be $2 \times 0.5 \times 0.5 = 0.5$. Similarly, for a bout consisting of three visits, the probability of all visits being to the same food type would be $0.5 \times 0.5 \times 0.5 = 0.125$. Likewise, the probability of two visits to one food type and one to the other food type would be $3 \times 0.5 \times 0.5 \times 0.5 = 0.375$. The same process can be repeated for bouts consisting of four or any other number of visits. In this manner the

frequency distribution of bout composition was constructed which could be expected for animals that did not regulate diet choice during a meal.

The same procedure can be followed for an experiment in which the proportion of observed visits to H feeders is, say, 0.7. The probabilities are then 0.7 and 0.3 that a randomly drawn visit is to an H or L feeder, respectively. For a two-visit bout the proportion of visits to an H feeder can be 0, 0.5, or 1. The probability of each of these occurring can then be calculated as $0.3 \times 0.3 = 0.09$, $2 \times 0.3 \times 0.7 = 0.42$ and $0.7 \times 0.7 = 0.49$, respectively. Similarly, for a three-visit bout the proportion of visits to an H feeder can be 0, 0.33, 0.67 or 1. The probability of each of these occurring can then be calculated as $0.3 \times 0.3 \times 0.3 = 0.027$, $3 \times 0.3 \times 0.3 \times 0.7 = 0.189$, $3 \times 0.3 \times 0.7 \times 0.7 = 0.441$ and $0.7 \times 0.7 \times 0.7 = 0.343$, respectively. In this manner a frequency distribution of bout composition was constructed that could be expected for animals that did not regulate diet choice during a meal.

For each experiment, observed frequency distributions of meal composition were compared with predicted bout composition to test for evidence of animals regulating diet choice within a meal. To avoid increasingly complicated calculations for very rarely occurring meals, consisting of large numbers of visits, the above procedure was repeated while increasing the number of visits per meal until at least 90% of all the meals in an experiment were included in the comparison.

This approach was used to test the following hypotheses. If animals ate randomly from the two foods (as expected for Exp. 1) then one would not expect the observed

frequency distribution of meals to deviate from the predicted bout composition (hypothesis 3). Alternatively, if animals regulate a non-random diet choice on a meal basis then the observed frequency distribution of meals would be expected to deviate from the predicted bout composition. More specifically, it was hypothesised that meals with a composition close to the long-term average diet choice were expected to occur much more frequently than predicted by randomly composed bouts. In contrast, it was expected that meals with a composition far from the long-term diet choice would occur much less frequently than predicted by randomly composed bouts (hypothesis 4). Chi^2 analyses was used to test for differences between recorded meal and predicted bout compositions. To avoid the disturbing effects on the Chi^2 analysis of classes with very low numbers of observations (Zar, 1996), frequencies were grouped using the same 11 classes which were arranged symmetrically about a diet choice of 0.5, in all experiments.

4.3.9. Testing the relationship between proportion of visits to, and intake from, feeders supplying high protein food

In theory, an animal that consumes a meal with a proportion of visits to an H feeder that is far from the long-term proportion of H selected in the diet can 'compensate' by consuming different amounts of food depending on the food type it is eating. For instance an animal that selects 2/3 H in the long-term, can still select 2/3 H during a meal even if the proportion of visits to the H feeders during that meal is only 1/2. This can be achieved by consuming twice as much food during visits to the H as opposed to the L feeders. Therefore, any sign that the proportion of H consumed

during a meal deviates systematically from the proportion of visits to feeders supplying H during the meal, would be evidence for diet choice on a meal basis. This possibility was investigated by first calculating the expected diet choice, assuming this was not regulated within a meal. Subsequently, differences between expected and observed diet choice were calculated and regressed on the proportion of visits to H feeders. The null hypothesis was that animals would attempt to regulate diet choice within a meal by compensating in this way (hypothesis 5). A significant negative regression coefficient would provide evidence for diet choice on a meal basis, whereas a non-significant regression coefficient would falsify the hypothesis.

4.4. Results and Discussion

4.4.1. General

Figure 4.1 shows the consistent nature of group mean diet choice in the three experiments. During the four weeks of Exp. 1, diet choice remained close to 500 g of H/kg of intake, i.e. cows apparently ate a random diet. In contrast, diet choice was consistently around 700 g of H/kg of intake during the 33 weeks of Exp. 2 and the two weeks of Exp. 3, i.e. clearly different from random intake. Table 4.2 gives a summary of the daily intake, number of visits and visit duration of the cows in each of the three experiments. It can be seen that the duration of visits differed considerably between Exp. 3 and those of Exp. 1 and Exp. 2. Part of these differences was probably related to differences in food composition between experiments. The higher silage to concentrate ratio in Exp. 3, compared to the other experiments, was associated with a higher average daily visit duration in this experiment. This is in agreement with observations by Friggens *et al.* (1998). The lower number of daily visits in Exp. 1 and Exp. 2, compared to Exp. 3, was very likely related to the presence of yokes which have been found to significantly affect the recorded visit characteristics, though not to affect the total daily intake (Tolkamp *et al.*, 2000). The total daily DM intake was similar for cows in all three experiments (Table 4.2).

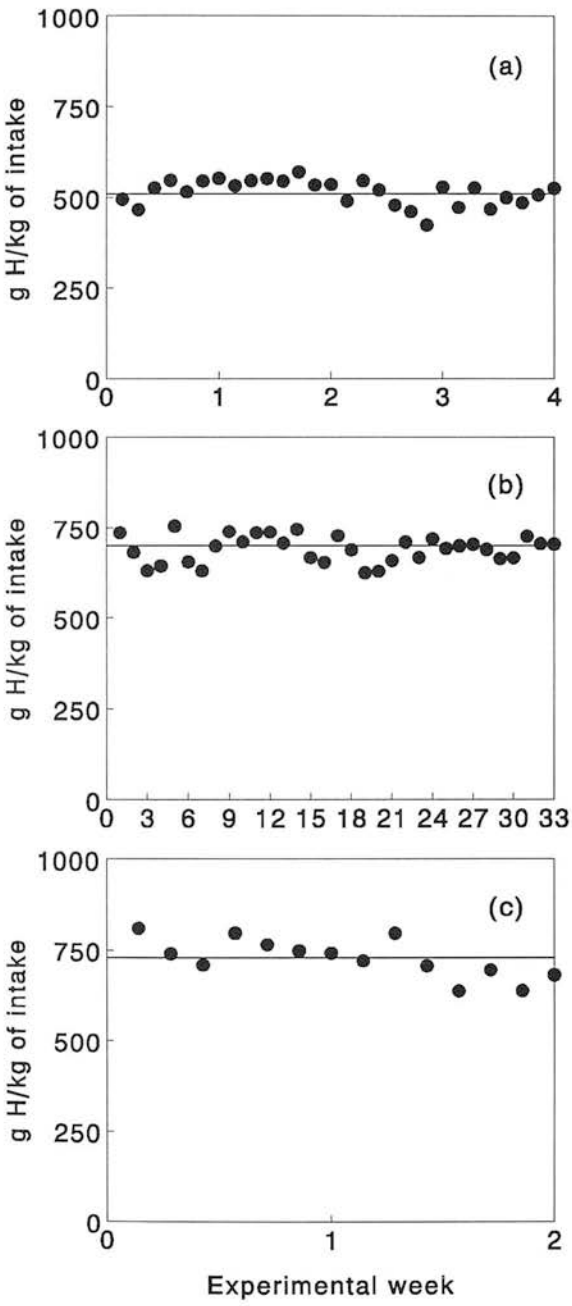

Figure 4.1. *Mean of individual daily (a and c) or weekly (b) diet choice. The solid line represents the long-term average diet choice. Figures a, b and c refer to experiments one, two and three, respectively.*

Table 4.2. *Average (± s.e.) of individual mean intake and number of visits*

	Experiment 1	Experiment 2	Experiment 3
Daily DMI (kg[a] DM)	21.0 ± 0.68	20.6 ± 0.34	19.7 ± 0.30
Daily FMI (kg[b])	46.5 ± 1.50	43.9 ± 0.74	47.9 ± 0.73
Daily number of visits	27.1 ± 2.52	31.9 ± 2.61	62.4 ± 6.68
Daily visit duration (h)	2.3 ± 0.09	2.3 ± 0.10	3.5 ± 0.17

[a]Dry matter.

[b]As fed.

4.4.2. Visit-based analyses

Table 4.3 shows that no aspect of the recorded visit characteristics from Exp. 1 (expressed as a proportion) differed from 0.5, i.e. none differed from random. This is in agreement with the visit characteristics presented in Table 4.4 which also shows that animals in this experiment did not consume a diet that differed from random. Therefore, as animals in Exp. 1 consumed a diet at random from the two foods, hypothesis 1 cannot be rejected. This confirms previous analysis of the diet choice for these cows based on daily intakes (Tolkamp *et al.* 1998c). Table 4.4 shows that within all experiments, feeding rate was unaffected by the food being consumed. Therefore, animals show no evidence of regulating choice by altering the rate at which they consume the foods. However, when comparing the feeding rate across experiments, the lower rate in Exp. 3 is evident. This is likely to be related to the higher silage to concentrate ratio in Exp. 3, compared to Exp. 1 and Exp. 2 (Tolkamp *et al.*, 2002).

Table 4.3. *Average (± s.e.) of individual intake, number and duration of visits to the high protein (H) food as a proportion of total intake, number and duration of visits*

	Experiment 1		Experiment 2		Experiment 3	
	Mean ± s.e.	*P*-value	Mean ± s.e.	*P*-value	Mean ± s.e.	*P*-value
Intake of H as a proportion of total intake[a]	0.51 ± 0.022	0.516	0.70 ± 0.015	<0.001	0.73 ± 0.018	<0.001
Number of visits to H feeders as a proportion of total number of visits[a]	0.50 ± 0.019	0.854	0.66 ± 0.010	<0.001	0.67 ± 0.020	<0.001
Duration of visits to H feeders as a proportion of total visit duration[a]	0.52 ± 0.022	0.366	0.70 ± 0.015	<0.001	0.73 ± 0.018	<0.001

[a]Means were tested to determine if they were significantly different from 0.5, i.e. random.

Table 4.3 shows that, in contrast to Exp. 1, all recorded visit characteristics from Exp. 2 and Exp. 3 differed from that expected if animals visited feeders at random ($P < 0.001$). Mean visit duration and consequently intake per visit (Table 4.4) were seen to be higher ($P < 0.001$) when visiting H feeders in Exp. 3, with the same (non-significant) trend recorded in Exp. 2 ($P = 0.055$). However, the recorded intake per visit to H feeders in Exp. 3 was only 23% higher than the intake per visit during visits to L feeders. If a diet choice of 0.73 H (Table 4.3) was achieved by having higher intake per visit at H compared to L feeders then the intake per visit at feeders supplying H would need to be 170% higher than at the L feeders. Clearly, the higher

intake per visit at H feeders contributed relatively little to regulating diet choice. Therefore, hypothesis 2 cannot be rejected, as cows appear to select their diet mainly by visiting H feeders more frequently than L feeders.

Table 4.4. *Average (± s.e.) of individual intake per visit, duration per visit and intake rate at the feeders supplying high (H) and low (L) protein food*

	Experiment 1			Experiment 2			Experiment 3		
	H1	L1	*P*-value	H2	L2	*P*-value	H3	L3	*P*-value
Mean intake per visit[a] (kg[b])	1.98 ± 0.060	1.89 ± 0.071	0.359	1.60 ± 0.062	1.41 ± 0.063	0.055	1.00 ± 0.022	0.81 ± 0.032	<0.001
Mean visit duration[a] (minutes)	6.08 ± 0.211	5.57 ± 0.206	0.190	5.03 ± 0.243	4.39 ± 0.228	0.081	4.14 ± 0.113	3.35 ± 0.161	<0.001
Mean feeding rate[a] (kg[b]/minute)	0.35 ± 0.008	0.37 ± 0.005	0.137	0.35 ± 0.008	0.34 ± 0.010	0.386	0.26 ± 0.004	0.25 ± 0.018	0.497

[a]Mean ± s.e. of individual feeder supplying the given food type, with cow effect accounted for.

[b]As fed.

4.4.3. Estimation of meal criteria

Meals generally consist of more than one visit and can thus be subject to analysis of diet choice. Before visits can be clustered into meals, meal criteria must first be estimated. Figure 4.2 shows the recorded frequency distribution of the $\log_e$-transformed interval lengths between visits, pooled across cows, and the fit of the model presented in Chapter 2. The observations consist of three populations; there is a peak at long intervals, and two overlapping peaks at shorter interval lengths. The peak at long interval lengths is associated with the long intervals between meals (Tolkamp *et al.*, 1998a), whereas the other peaks represent within meal intervals when cows do, or do not, visit the drinker (Tolkamp and Kyriazakis, 1999b). The meal criterion is estimated at the point where these distributions cross. Figure 4.2 shows there is little overlap between the final two populations, therefore the meal criterion can be reliably determined as few intervals will be assigned to the wrong population (Chapter 2). Table 4.5 gives the average meal criteria for the cows in each of the experiments. The estimated meal criteria were similar for cows in all three experiments and were comparable with estimated meal criteria found by Tolkamp *et al.* (2002). The frequency distribution of interval lengths (Figure 4.2) from Exp. 3 differs from the other experiments as it contains a smaller proportion of long intervals. This is a result of the higher daily number of visits in this experiment (Table 4.2) and, consequently, there is a lower proportion of between meal intervals.

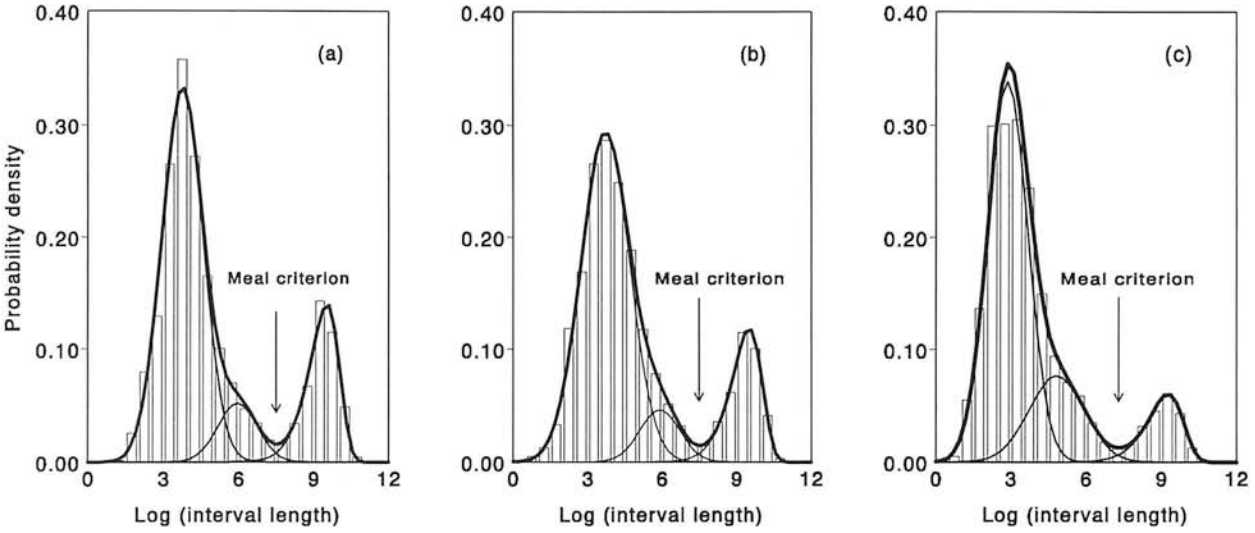

Figure 4.2. *The probability density function for the pooled observations from experiments one, two and three (a, b and c, respectively). Thin lines represent the contribution of each population to the total probability density (thick line). The bars represent the observations (relative frequency divided by class width, i.e. 0.5 $\log_e$ units). The first population (i.e. with the shortest mean interval length) represents intervals within meals in which the cows did not drink. The second population represents intervals within meals in which the cows did drink. The third population represents the between meal intervals.*

4.4.4. Meal-based analyses

Table 4.5 gives a summary of the meal characteristics for the three experiments. The mean number of meals each day was remarkably similar between experiments and across cows, considering the large difference in mean number of visits per day (Table 4.2). This is in agreement with Tolkamp *et al.* (2000) where the meal was

found to be a more biologically relevant unit of intake than the visit. Intake per meal was also similar between experiments, however meal duration was greater in Exp. 3 compared to Exp. 1 and Exp. 2. This was a consequence of both the decreased intake rate (Table 4.4), which was probably linked to the high silage to concentrate ratio, and the greater number of visits per meal in this experiment. Therefore, as meal duration is a summation of time spent visiting the feeders and the duration of within meal intervals, the increased number of visits lead to an increased number of intervals and hence contributed to a greater meal duration.

Table 4.5. *Average (± s.e.) of individual meal criteria and individual mean meal characteristics*

	Experiment 1	Experiment 2	Experiment 3
Meal criterion (minutes)	35.5 ± 3.27	25.6 ± 1.47	26.9 ± 2.06
Daily number of meals	5.7 ± 0.26	6.0 ± 0.21	6.6 ± 0.75
Intake per meal (kg[a])	8.2 ± 0.41	7.3 ± 0.29	7.3 ± 0.32
Meal duration[b] (minutes)	33.0 ± 1.75	34.3 ± 1.75	42.2 ± 2.36

[a]As fed.

[b]Sum of time spent feeding and non-feeding intervals within a meal.

The recorded long-term consistency in diet choice (Figure 4.1) could be a result of animals regulating diet choice within meals by composing many meals with a diet choice that approximates the long-term diet choice. Alternatively, the long-term consistency in diet choice could be the result of 'averaging out' over multiple meals with a wide range of compositions. If the meal is the unit of diet choice regulation

then during Exp. 2 and Exp. 3 one would expect to see many meals with a composition of approximately two thirds of the visits to the H feeders. Animals would be expected to avoid eating meals consisting of one food type only as the diet choice resulting from such meals is far from the long-term average. However, as cows in Exp. 1 ate a random diet one would expect to see, in this experiment, a frequency distribution of meal and of bout composition that did not differ.

4.4.5. Meal-based analyses - Experiment one

Figure 4.3a compares the distribution of recorded meal compositions (up to nine visits per meal) and predicted bouts. There is a remarkable similarity between the frequency distribution of observed meal and predicted bout compositions. However, contrary to the expectations, cows had a higher frequency of single food type meals than predicted from the frequency distribution of bout compositions (Figure 4.3a). Associated with this, the observed frequency of meals with a composition similar to the long-term average was lower than expected. This resulted in a significant difference between the frequency distribution of recorded meals, and predicted bouts ($\chi^2 = 104$, df = 10, $P < 0.001$). Prior analysis of diet choice based on daily intakes (Tolkamp *et al.*, 1998c), and the visit-based analysis (shown above) strongly suggested that cows in this experiment ate randomly. However the results from the meal-based analysis (Figure 4.3a) did not agree with this. In principle, at least two types of explanation could account for this apparently non-random behaviour. The cows could be regulating their diet choice, despite avoiding meals with a composition similar to the long-term average diet composition. However, the

evidence discussed above showed this was not the case. Alternatively, this discrepancy could be the result of an error in the assumptions used to predict bout composition. Therefore the feeding data from these cows was examined more closely to determine what might have caused this result.

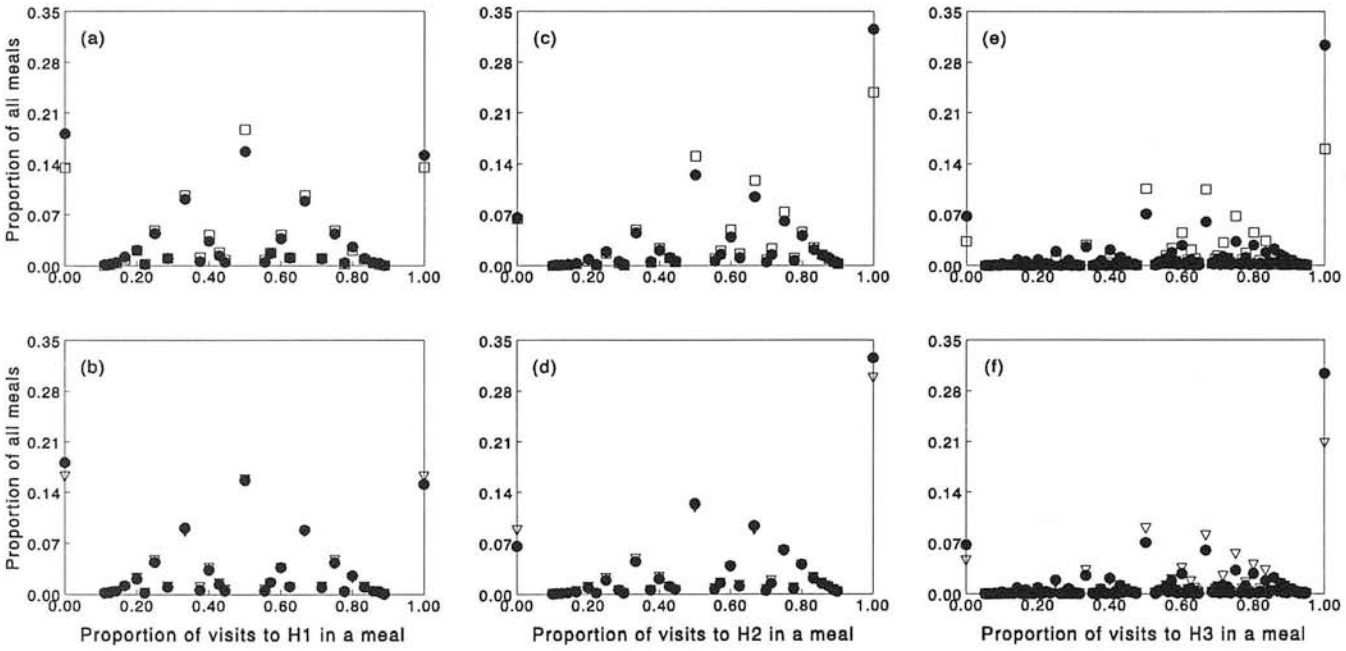

Figure 4.3. *The proportion of all meals that have a given proportion of visits to feeders supplying high protein (H) food in a meal. Figures a, c and e give the frequency distribution of the predicted bout composition (open squares) if bouts are composed at random from the observed visits in experiment one, two and three, respectively. Figures b, d and f give the frequency distribution of the adjusted predicted bout composition (open triangles) if bouts are composed at random, having adjusted for revisits to the same feeder, from the observed visits in experiment one, two and three, respectively. The recorded frequency distribution of meal compositions is given as solid dots.*

The frequency distribution of bout composition in Figure 4.3a was predicted on the assumption that the probability of a cow visiting a given feeder was constant. For Exp. 1, the probability of visiting each food type was 0.5 (Table 4.3) and there were six feeders per food type. These predictions were based, therefore, on the assumption that the likelihood of a cow visiting a feeder was $0.5 / 6 = 0.083$ for each feeder. This likelihood was assumed to be independent of the feeder the cow visited previously. The records showed, however, that this was not the case. The probability of a cow re-visiting the feeder it had just left was, in fact 0.23, i.e. much higher than expected from the assumption of randomness. Revisits were observed to a similar extent for H and for L feeders. The propensity of cows to re-visit the same feeder was affected by time of the day. The highest incidence of re-visits to the same feeder within a meal were recorded during periods of high feeding activity (e.g. immediately after fresh food supply) and the lowest during quiet periods (i.e. at night).

Observations of cows in the experimental facility described above have shown that cows may be disturbed by other cows whilst visiting a feeder. Cows were seen to leave the feeder momentarily, then re-enter the same feeder where they continued to feed. Therefore, it seems likely that the higher probability of cows revisiting the same feeder is related to this disturbance of feeding animals. Since revisits to the same feeder imply another visit to the same feed type, this higher likelihood can be expected to result in a higher frequency of single food type meals than if the probability of visiting all feeders is assumed to be equal. At the same time, this would decrease the relative frequency of meals with mixed composition, as observed in the data (Figure 4.3a).

The expected frequency distribution of bout compositions was re-calculated, taking into account this higher probability of revisits to the same feeder (see Appendix B). Figure 3b shows the adjusted frequency distribution of bout composition. Differences between recorded meal and predicted bout compositions seen in Figure 4.3a, have largely disappeared and are no longer significant (χ^2 = 15, df = 10, $P > 0.1$). Therefore hypothesis 3 cannot be rejected because, when the higher probability of revisits to the same feeder was taken into account, the bouts predicted on the basis of random behaviour did not differ from the actual meals composed by cows in Exp. 1.

The results from Exp. 1, where cows ate at random, were therefore used to test the underlying assumptions used to predict bout composition. This led to the development of a refined methodology to calculate bout composition, taking into account revisits to the same feeder. This methodology was used to predict the bout compositions for Exp. 2 and Exp. 3 (see below). The hypotheses, presented above, could then be tested with confidence in the assumptions used to predict bout composition.

4.4.6. Meal-based analyses - Experiment two

Figure 4.3c compares the distribution of recorded meal compositions (up to ten visits per meal) and predicted bouts. The remarkable similarity between recorded meal, and predicted bout compositions was again evident. The observations are clearly skewed toward meals with a proportion of visits to the H feeders that reflects the long-term

diet choice (Table 4.3). However, there are less of such meals than predicted by the composition of bouts. Again, there is a corresponding excess of single food type meals. This difference between the frequency distribution of observed meals and predicted bouts was significant ($\chi^2 = 1030$, df = 10, $P < 0.001$). Examination of the feeding patterns of these cows revealed an elevated probability of a revisit to the same feeder, as observed in Exp. 1. The expected meal compositions were therefore adjusted using the methodology developed with data from Exp. 1, using the proportion of revisits found in Exp. 2 (0.32). Figure 4.3d gives the recorded meal and adjusted predicted bout compositions. The differences between recorded meal and predicted bout compositions seen in Figure 4.3c have almost disappeared, but are still significant ($\chi^2 = 137$, df = 10, $P < 0.001$). Figure 4.3d shows that this significant difference was largely the result of differences, between recorded meal and predicted bout compositions, for meals and bouts composed of visits to one food type only. The frequencies of meals and of bouts, with a composition similar to the long-term average diet selection, were approximately equal. If cows regulated diet choice within meals then one may expect to see a very high frequency of meals with a composition that is similar to the long-term average diet choice. As this was not the case, the analysis provides no evidence that animals regulate diet choice on a meal basis. Therefore, hypothesis 4 must be rejected on the basis of Exp.2.

4.4.7. Meal-based analyses - Experiment three

Figure 4.3e compares the distribution of recorded meal compositions (up to 19 visits per meal) and predicted bouts. The discrepancy between recorded meals and

predicted bouts can be seen, again there are many more single food type meals than expected. This resulted in a significant difference between the frequency distribution of recorded meals and predicted bouts ($\chi^2 = 1365$, df = 10, $P < 0.001$). Examination of recorded feeding behaviour showed that, also in this experiment, revisits occurred more than would be expected on the basis of randomness. The expected meal compositions were therefore adjusted using the method developed in Exp. 1, using the proportion of revisits found in Exp. 3 (0.27). Figure 4.3f gives the frequency distribution of recorded meal and adjusted predicted bout compositions. The difference between these frequency distributions is still considerable even after correcting for revisits ($\chi^2 = 317$, df = 10, $P < 0.001$). Therefore there is no evidence that cows in Exp. 3 attempt to compose many meals with a composition that reflects the long-term diet composition, indeed there are more meals whose composition is far from the long-term diet composition than predicted. Therefore, hypothesis 4 must be rejected on the basis of Exp. 3 also.

4.4.8. The relationship between proportion of visits to, and intake from feeders supplying high protein food

Figure 4.4 shows the observed average diet choice per meal in relation to the proportion of visits to feeders supplying H in the meal. The graphs also show the long-term average diet choice. If animals attempted to regulate diet choice within a meal by adjusting their intake per visit, then the observations would have clustered around this long-term average. It is evident that this did not happen in any of the experiments. Instead, the observations are clustered around the expectations for

animals that do not regulate diet choice on a meal basis. Indeed, regression analysis confirmed that differences between observed and expected diet choice was not affected by the proportion of visits to H feeders in the meal ($P > 0.6$, 0.8, 0.5, for Exp. 1, Exp. 2 and Exp. 3, respectively). Therefore, the analysis provides no evidence that cows regulate diet choice within a meal. Thus hypothesis 5 must be rejected.

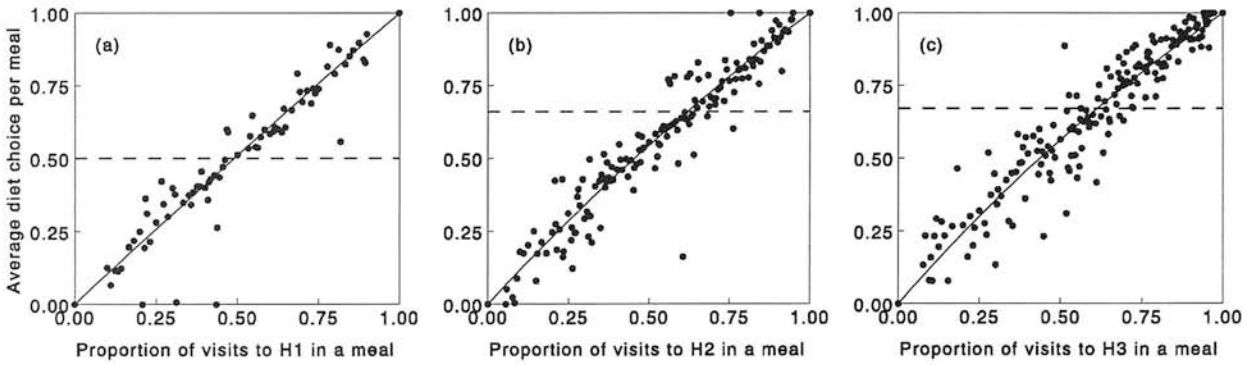

Figure 4.4. *Average diet choice (g of high protein (H) food consumed per kg of total intake in the meal) in relation to the proportion of visits to H feeders in the meal. Figures a, b and c indicate experiments one, two and three, respectively. Dots represent the observations. The broken line is the expected diet selected if diet choice was regulated perfectly on a meal basis (i.e. equal to the long-term diet choice). The solid line represents the expected diet choice calculated on the assumption that animals did not adjust the amounts eaten per visit according to the proportion of visits to feeders supplying high protein food during a meal. The slight curvature of the solid line is a result of the higher average intake per visit to the feeders supplying high, compared to low, protein feed.*

4.5. General Discussion

Diet choice experiments, in which animals have access to two similar foods that differ in one nutrient dimension (e.g. protein to energy ratio), can be useful research tools to investigate a number of questions. Such experiments can allow animals to demonstrate their requirements for a given nutrient ratio in the long-term (Tolkamp *et al.*, 1998b). To that end, experiments entailing longer-term observation of the diet selected when animals are given a choice of foods have been carried out with many species (e.g., pigs: Kyriazakis *et al.*, 1990; sheep: Kyriazakis and Oldham, 1993; poultry: Forbes and Shariatmadari, 1996; cows: Tolkamp *et al.*, 1998b). The conclusion of some studies has been that animals do not always select a diet that allows them to perform as well as animals fed a single diet (Galef, 1991; Larue-Achagiotis *et al.*, 1992). However, the conclusion of most such experiments is that animals are indeed able to select an appropriate and consistent diet over long periods of time (Forbes, 1995a; Rovee-Collier *et al.*, 1996; Kyriazakis and Emmans, 1999).

There usually is variation in diet selection between individuals (Tolkamp and Kyriazakis, 1997; Atwood *et al.*, 2001). For analyses using probability theory, however, large data sets are essential in order to produce reliable results; i.e. pooling of individual data is frequently required. In theory, pooling of diet choice information obtained with individuals could lead to erroneous conclusions (Chapter 3). For instance, some cows could have often attempted to select a diet within a meal that was similar to the long-term average diet choice whereas others could have typically eaten meals consisting of one food type only. Such different feeding

strategies would result in non-normal frequency distributions of individual SD's of meal average proportion of visits to feeders supplying H. In such cases, the pooling of data obtained with individuals could have led to inappropriate rejection of the hypotheses. However, in all experiments these distributions were normal, which shows that cows did not have greatly differing behavioural strategies. Therefore, the results were not affected by pooling of data across individuals.

Analyses of diet choice during short-term feeding behaviour may also inform one of the relevant time scale over which diet choice is regulated and nutrient supply is synchronised. Recently, there has been a lot of interest in the synchronisation of energy and protein supply (Sinclair *et al.*, 1993; Forbes and Shariatmadari, 1994; Witt *et al.*, 1999b). Since animals with access to two foods can select when to eat and which food to consume, such analyses may reveal how important the animal perceives synchronisation to be. In addition, better knowledge of the time scale of diet choice may enable the deduction of likely mechanisms of nutrient monitoring and hence the way diets are selected.

Analyses of the short-term feeding behaviour of cows that do select a consistent non-random diet over prolonged periods of time, provided no evidence that animals attempted to reach a diet composition within a meal that is consistent with their longer-term average preferences. This might be related to the nutritional dimension of the foods that cows monitor and therefore use to select a diet. From previous analyses it was concluded that cows very likely select for eRDP rather than for MP (Tolkamp *et al.*, 1998c). In the experiments analysed here, the concentration of

eRDP differed between H2 and L2, and between H3 and L3, but was similar for H1 and L1. This coincides with when cows did, and did not, select a non-random diet. Therefore, although the foods in Exp. 1 differed in a nutrient dimension (Table 4.1) this did not affect the cows' feeding behaviour. However, the cows in Exp. 2 and Exp. 3 may have selected a diet based on the eRDP supply of the foods.

Effective rumen degradable protein is a measure of the protein that is available for microbial degradation (McDonald *et al.*, 2001). Such degradation results in ammonia release in the rumen (Parker *et al.*, 1995). Different protein sources are degraded by microbes at different rates (Parker *et al.*, 1995) resulting in the ammonia in rumen fluid peaking at around one to six hours after feeding (e.g., Church, 1969; Al-Rabbat *et al.*, 1970; Nicholson *et al.*, 1992). Therefore regulating diet choice, by monitoring differences in eRDP between foods, may not be possible within the meal. For this to occur, feedback from the food consumed in meals, which are typically 30 to 50 minutes in duration (Table 4.5), may be insufficient to allow the diet composition to be altered within such a short time frame. However, animals can learn the nutritional consequences of feeding behaviour (Collier and Johnson, 1990; Forbes, 1995a; Provenza, 1995). Therefore, animals may regulate their protein intake within a meal to ensure synchronisation of protein to energy ratio in the subsequent inter-meal interval, i.e. the next few hours. However, no evidence was found that animals attempt to synchronise their diet over this time span.

In contrast to monogastrics, when ruminants eat a meal they add food to a rumen that contains the remnants of previous meals. The immediate metabolic consequences of

the addition of a single meal may therefore be affected by the animal's previous nutritional history. Therefore, the relevant time period of regular diet choice may be greater than a single meal. Indeed, Tolkamp and Kyriazakis (1997) found that some cows selected a lower than average proportion of H feed during peak, i.e. maximum competition, compared with non-peak periods. This is in agreement with Harb *et al.* (1985) where subordinate cows were found to alter their feeding patterns such that they achieved a daily intake similar to dominant cows. Subordinate cows may therefore have been compensating at non-peak times for a low protein intake during the peak periods, when access to H feeders may have been limited. The differences in intake patterns between animals, observed by Tolkamp and Kyriazakis (1997), was likely due to differences in cow pressure per feeder between H and L feeders. Changes in cow pressure can greatly influence the feeding behaviour of cows (Elizalde, 1993; Elizalde and Mayne, 1993; Tolkamp *et al.*, 2000). This situation was similar to that of Exp. 3, where cow pressure per feeder was higher at H, compared to L feeders. Therefore attempts to compensate for the effects of cow pressure might explain the differences in meal composition seen between Exp. 2 and Exp. 3. Subordinate cows in Exp. 3 may have had limited access to H feeders during peak intake periods. They may have compensated for this at non-peak periods by eating meals consisting predominantly of H. This could then have led to the elevated level of single food type meals seen in Figure 4.3f. This suggests that cows may have attempted to regulate diet choice during longer time spans, e.g. within a day.

Other evidence, however, suggests that the relevant time span for obtaining an appropriate mixture of nutrients may be longer than a day. For instance, cows that

were given alternate access to high and low protein food for three days at a time did not show any decline in intake or milk yield, while such a decline was evident after a week for cows with access to low protein food only (Tolkamp and Kyriazakis, 1997). This suggests that lactating cows can tolerate an asynchronous diet for several days, but not for a week. Work with much smaller animal species (e.g. chickens) strongly suggest that animals can tolerate asynchronous supplies of energy and protein for periods of more than one day (Forbes and Shariatmadari, 1996). It must be noted that experiments such as these provide evidence of an animal's ability to tolerate nutritional imbalances (Bigelow and Houpt, 1988). However, these do not suggest the time period over which animals regulate diet composition when they are given a free choice. However, if such small and metabolically more intensive species (Kyriazakis *et al.*, 1999) can tolerate a diet, which is far from their long-term diet choice, for such periods of time then the conclusion that for cows the relevant time span exceeds a meal may not be surprising.

Although observations on the effects of synchronicity of nutrient supply are not all consistent (i.e., Nia *et al.*, 1995; Shabi *et al.*, 1998; Kim *et al.*, 1999b), the conclusion of a review (Chamberlain and Choung, 1995) of such work for dairy cows concludes that animals are very flexible and can overcome considerable time spans with an asynchrony of nutrient supply. The work presented in this chapter seems to agree with this conclusion, as no evidence was found that cows attempted to select a diet similar to the long-term average within a meal. Ruminants have evolved to graze in heterogeneous environments, therefore they may be expected to be adapted to some asynchrony in the supply of nutrients. Perhaps an asynchronous nutrient supply can

be tolerated for periods up to a few days. Present analysis does not suggest what the most relevant time scale is, except that is must be longer than a meal. In addition, time may not be the dimension over which animals regulate diet choice. Other dimensions that animals may use include monitoring the deviation of nutrient supply from a desired level (i.e. some measure of nutrient intake), or the number of randomly composed meals that will be tolerated before the diet composition is corrected back to within physiologically determined margins. Therefore, as animals are quite flexible, they might not react to an asynchronous nutrient supply as long as the selected diet has a protein content within certain physiological margins.

The type of data presented in this study is therefore considered potentially suitable for performing analyses in order to answer such questions. For instance, the effects of the diet composition during the preceding meal, or during a longer given time span, on the diet selected during the subsequent meal can be analysed. Therefore, large data sets of choice fed animals allow further investigation of the relationships between short-term meal composition and long-term diet selection. This may provide an insight into the relevance for animals of synchronisation of nutrient supply.

CHAPTER FIVE

Analysing the structure of short-term feeding behaviour: Deterministic vs. probabilistic control of food intake regulation

5.1. Abstract

Despite many years of study, the underlying mechanisms which animals use to regulate their food intake remain unclear. Determination of whether short-term feeding behaviour is better described as deterministic or probabilistic in nature may shed light on the mechanisms animals use to regulate food intake. To this end, the following objectives were investigated using data from a large diet choice experiment where 16 diary cows were offered a choice between high and low protein foods and their feeding behaviour was recorded electronically. First, the prediction of the satiety concept, that the probability of a meal ending would increase with time and/or amount eaten in the meal, was assessed. Secondly, the diurnal pattern of intake and diet composition was calculated, using sine wave analysis, to determine if there was evidence of animals attempting to maintain a consistent diurnal pattern in their feeding behaviour. This could be indicative of regulation of feeding behaviour in the short-term. Finally, the historical use of prandial correlations, in support of short-term regulation of feeding behaviour, was addressed. The findings of this work were firstly that the short-term feeding behaviour of cows is well predicted by the satiety concept. Therefore, in line with predictions from this concept, as a meal progresses, i.e. as more food is eaten and the meal duration increases, cows have an increasing probability of ending a meal. In contrast, the probability of ending a visit is not in agreement with the predictions of the satiety concept, as the ending probability does not increase with time or amount eaten. Secondly, animals were shown to have a diurnal pattern in their feed intake, with greater intake during feeding cycles (i.e., the period from the end of one meal until the end of the

subsequent meal) beginning in the day. In contrast, there was no consistent diurnal pattern in the composition of the diet selected. Thirdly, the relationship between the length of the pre-meal non-feeding interval and the size of the meal (pre-prandial correlation) and the length of the post-meal non-feeding interval and the meal (post-prandial correlation) was not found to be biologically relevant. Previous conclusions, that prandial correlations may account for the feeding behaviour of animals, were questioned in light of this evidence. In conclusion, no evidence was found in support of previous conclusions in the literature that animals regulate their feeding behaviour in a short-term deterministic way. Indeed, the evidence suggests that animals are flexible in their short-term feeding behaviour and that this is better described as probabilistic in nature.

5.2. Introduction

Since the amount, and composition, of the diet that animals consume greatly affects their reproductive fitness it is likely that animals have evolved mechanisms with which to regulate their intake (Yearsley *et al.*, 2001). However, at present the nature of food intake regulation mechanisms remains open to debate (e.g., Conrad, 1966; Forbes, 1977b; Le Magnen, 1985; Illius and Gordon, 1991; Ketelaars and Tolkamp, 1992a,b; Mertens, 1994; Forbes, 1995a,b; Fisher, 1996; Mertens, 1996; Poppi, 1996; Farnsworth and Illius, 1998; Illius *et al.*, 1999; Kyriazakis *et al.*, 1999; Pittroff and Kothmann, 1999; Shipley *et al.*, 1999; Fisher, 2002). Because the diet that animals consume must, at least formally, be the result of the foods eaten in the short-term then the study of short-term feeding behaviour may provide information on how animals regulate their diet composition and intake in the long-term (Simpson, 1982; Forbes, 1985; Dürst *et al.*, 1993; Dado and Allen, 1994; Gill and Romney, 1994; Shorposner *et al.*, 1994). Such analysis can explore issues relating to the timeframe of diet regulation by determining if animals regulate their diet in the short-term and therefore support the notion of deterministic control of intake and diet selection. Such a strategy would enable animals to maintain a constant supply of nutrients and hence potentially maximise the efficiency of nutrient use within the short-term. The alternative would be that the short-term feeding behaviour of animals is more representative of probabilistic behaviour indicating regulation within the medium-term and hence more flexible short-term feeding behaviour.

The first issue to be explored within this chapter has been identified as a result of work conducted within Chapter 2, where a model describing the frequency distribution of intervals between visits to the feeder was presented. This model led to the estimation of a meal criterion, which enabled the observed feeding behaviour, i.e. visits to the feeder, to be clustered into meals in a biologically sensible way. Feeding behaviour could therefore be explored in terms of meals, which have been proposed as a more biologically relevant unit of intake regulation than visits (Tolkamp *et al.*, 2000). This model was based on the implicit assumption that the feeding behaviour of animals was described by the predictions of the hunger and satiety concept (Le Magnen, 1985). This concept predicts that as the non-feeding time since the last meal increases then animals will become more hungry, therefore increasingly likely to initiate a meal. Evidence in support of this prediction has been presented in Simpson and Ludlow (1986), Tolkamp and Kyriazakis (1999), Chapter 2, and Chapter 3. The satiety concept also implicitly assumes that as animals consume food during a meal then they become more satiated. Consequently the probability of an animal ending a meal increases as meal duration and/or intake increases. Such analysis of ending probabilities may provide greater insight into how the mechanisms of satiation operate. Therefore, the first objective of this chapter was to test the hypothesis that the probability of animals ending a meal would increase with time and/or amount eaten and to contrast this with the observed change in visit ending probability. Further to this, pooling data across day and night or individuals was found to influence the calculated probability of starting meals in Chapter 3. Therefore, this work was extended within the current chapter to explore the consequences of pooling data for the interpretation of meal and visit ending probabilities. Such analysis can

highlight how the probability of ending visits and meals changes with time and intake since the start of the meal. If feeding behaviour is controlled in the short-term then characteristic patterns of behaviour would be expected where ending probabilities display marked changes after a given level of intake. This analysis therefore provides new information with which to explore the predictions of the satiety concept and hence better understand the regulation of short-term feeding behaviour.

Analyses of short-term feeding behaviour in Chapter 3 showed that there was diurnal variation in the feeding behaviour of cows with more meals, and hence more food, eaten during the day. This is in agreement with the findings of Tolkamp *et al.* (2002) where feed intake of cows was found to follow a sine wave pattern with higher intakes during the day and lower intakes during the hours of darkness. When cows are offered a choice of two foods, one with a higher and one with a lower protein content, then the diet selected, as well as the quantity eaten, has been shown to vary diurnally (Tolkamp and Kyriazakis, 1997). In the experiment reported by Tolkamp and Kyriazakis (1997) the diet selected was shown to contain less of the high protein food during periods when the feeders were busy (mostly during the day) and have a greater proportion of this food when few cows were feeding (mostly at night). This diurnal pattern of diet composition is of interest because if the biological basis of such a pattern can be identified then this may improve our understanding of the mechanisms used by animals to regulate their diet. Additionally, if the pattern of diet regulation shown by animals is repeatable over time and is shown not to vary greatly then this may indicate a short time frame of diet regulation. In light of the findings of

Tolkamp and Kyriazakis (1997) it was therefore hypothesised that the feeding behaviour data of the cows analysed within this chapter would show a clear diurnal pattern in diet composition, with a lower protein diet selected during the day then the night. Further, if cows attempt to maintain a constant daily pattern in their diet composition then limited variation about the observed pattern of diet choice would be expected. The second objective of this chapter was therefore to determine how both intake and diet composition varied through the day and night by using sine wave analysis in order to determine if there was evidence for animals regulating their diet in the short-term.

Historically, the calculation of prandial correlations has been used to investigate intake regulation (Slater, 1974; Savory, 1981; Le Magnen, 1985; Bigelow and Houpt, 1988) and the presence of significant correlations has been taken as evidence of short-term intake regulation (Le Magnen and Devos, 1980; Schilstra, 1981). Prandial correlations have been calculated as the correlation between the intake in a meal and the preceding non-feeding interval (pre-prandial correlation) and the correlation between the intake in a meal and the length if the non-feeding interval to the next meal (post-prandial correlation). There has, however, been controversy both over the significance of these correlations (De Castro, 1975; Simpson, 1982; Bigelow and Houpt, 1988; Collier and Johnson, 1990) and how they should be calculated (Demaria-Pesce and Nicolaidis, 1998). Indeed, it has been questioned whether prandial correlations are ever evident, or whether their existence is a result of inappropriate analysis (Panksepp, 1973). A data set that was considerably larger than those typically used in the literature was therefore analysed in order to test the

hypothesis that feeding behaviour would be regulated in the short-term and that his would be indicated by the presence of strong prandial correlations. Such analysis can therefore provide evidence with which to address the many conflicting reports regarding the biological significance of prandial correlations.

The results from this work will shed new light on the issue of food intake regulation by addressing the question of whether animals regulate their intake in a short-term deterministic way or whether short-term feeding behaviour is better described as probabilistic in nature. Therefore, during this chapter the results from analysis of a large diet choice experiment are used to test hypotheses about how animals will regulate their diet within the short-term.

5.3. Materials and Methods

5.3.1. General

This study utilised data collected during the diet choice experiment described by Tolkamp *et al.* (1998b). Briefly, the experiment took place at the Langhill Dairy Cattle Research Centre (Edinburgh, Scotland). Sixteen Holstein-Friesian cows were kept in a yard, as described by Tolkamp and Kyriazakis (1997), for the duration of the experiment (156.5 ± 12.9 days per cow). The yard was equipped with 28 computerised food dispensers (Insentec B.V., Marknesse, The Netherlands) as described by Tolkamp *et al.* (1998a). Each food dispenser consisted of a bin with a capacity of 160L, mounted on two load cells. Access to the bin was via a pneumatically operated gate, controlled by a computer. The gate was equipped with an antenna that responded to the transponder that each cow wore around her neck. On entry to a feed bin, the cow's identification number, the weight of the bin and the time was recorded. When the cow left the feeder, the gate closed and the time was again recorded. Time was measured to the nearest second, and weight to the nearest 0.1kg. Access to the feeders was continuous except during milking, which lasted for up to 60 minutes (between 06:00-08:00 and between 16:00-17:30), and between about 08:00 and 09:30 when food residues were removed from the bins and fresh mixed food was supplied. Approximately three-quarters of the daily food was offered in the morning, with the remaining food added to the bins during the afternoon milking. The quantities of foods offered were calculated daily to allow at least 10%

refusals. Water was available *ad libitum* from two troughs situated one near each end of the row of food dispensers.

5.3.2. Foods and feeding behaviour

Foods were a mixture of 70% grass silage and 30% concentrate on a fresh weight basis and were formulated to have similar nutritional properties except for their protein contents, which were either high (**H**) or low (**L**). Table 5.1 shows that differences in the concentrate components of the foods resulted in two foods that differed in effective rumen degradable protein (**eRDP**) and metabolisable protein yield (MP).

Feeding behaviour data were collected, during 33 weeks. All cows had access to six H and six L feeders and 79,386 visits were recorded. The L feeders could also be accessed by some non-experimental cows, resulting in the cow pressure per experimental feeder being kept approximately equal for both food types (1.85 cows/feeder).

5.3.3. Clustering visits into meals

The visit was the shortest unit in which intake was recorded, however visits were generally clustered into meals. Calculation of a meal criterion allows the clustering of recorded visits into meals in a repeatable way. A meal criterion is an estimate of

the longest non-feeding interval, between visits to a feeder, which can be considered part of a meal (see Chapter 2). The lengths of intervals, between visits to the feeders, were calculated for each cow and were used to estimate individual meal criterion according to the mixed distribution methodology presented in Chapter 2. After calculation of individual meal criteria, visits were clustered into meals for each cow.

Table 5.1. *Experimental foods.*

Foods[a]	H	L
DM	465	464
CP	185	131
MP	114	76
ERDP	119	87
ME	12.3	11.8
FME	10.4	10.1
NDF	344	332

[a]Dry matter (DM) (g/kg), crude protein (CP) (g/kg DM) and neutral detergent fibre (NDF) (g/kg DM) were determined directly from chemical analysis of the foods (see Tolkamp and Kyriazakis, 1997). Metabolisable protein (MP) (g/kg DM), effective rumen degradable protein (eRDP) (g/kg DM), metabolisable energy (ME) (MJ/kg DM), and fermentable metabolisable energy (FME) (MJ/kg DM) were determined from chemical analysis and tabulated values for ingredients used (AFRC, 1993).

5.3.4. The probability of cows ending a visit or a meal

The probability of cows ending a visit, in relation to time since the start of the visit, was calculated from the observations in a similar way to the probability of starting a

meal, as presented in Chapters 2 and 3. Therefore, the proportion of visits with a duration > y minutes and ≤ y + 1 minute was divided by the proportion of visits with a duration > y minutes. Ending probabilities were also calculated in relation to amounts consumed as the proportion of visits with intakes of > z kg and ≤ z + 0.5 kg, divided by the proportion of visits with intakes of > z kg. These calculations were repeated for the probability of cows ending a meal in relation to time (averaged every two minutes) or intake (averaged every one kg) since the start of the meal. Ending probabilities were calculated for individuals separately and for all data pooled in order to further investigate the consequences of data pooling in light of findings presented in Chapter 3. After examination of the median interval lengths between meals in relation to the time of day (according to the methodology of Morgan *et al.* (2000b), see Chapter 3) all meals or visits that started between 08:00 and 20:00 were defined as occurring during the day, and as occurring in the night otherwise. Ending probabilities were then calculated for the day and the night separately. Ending probabilities, in relation to both time and intake, were calculated (with pooled data) for visits and for meals in which cows visited only one food type. To avoid random fluctuations in the ending probability due to low numbers of observations, the ending probabilities were only calculated if the number of visits or meals longer than y minutes, or greater than z kg, was more than 100.

5.3.5. Diurnal feeding pattern analysis

The diurnal patterns of feeding behaviour were explored by calculating how intake per hour of the feeding cycle and diet choice changed over the 24h day. The feeding

cycle was defined as the period from the end of one meal until the end of the subsequent meal. The diurnal patterns of intake per hour of the cycle or diet choice within a given cycle were investigated relative to the start time of the cycle. The fit of a sine wave model to the patterns of feeding behaviour over the 24h day was explored by fitting the following model, using least squares:

$$Y_i = a_1(1 + a_2\text{SIN}((2\pi/24)(t_i - a_3))) + e_i$$

Where Y_i = intake per hour of the feeding cycle i (kg/h) or diet choice in feeding cycle i (kg H/kg of intake), a_1 = mean level of the sine wave, a_2 = proportional amplitude of the sine wave, i.e. the amplitude as a proportion of the meal level of the sine wave, a_3 = shift in the sine wave (h), i.e. the pattern of the sine wave in relation to time, t_1 = start time of the feeding cycle i (h), and e_i = error term. The model was fitted both to individual animal data and to data pooled across animals. Sine wave models were fitted using programs written in Fortran 90 (Ellis *et al.*, 1994), which utilised NAG library routine E04 JAF (NAG, 1993). For graphical presentation, observed averages were calculated for the pooled data by grouping together all cow days in which a given number of meals were taken. Mean intake per hour of the cycle, or diet choice, and cycle starting times were calculated and plotted for each of the meals in days with between 4 and 8 meals as these accounted for 95% of all cow days.

5.3.6. Calculating prandial correlations

Prandial correlations were calculated from linear regression of the duration of the pre-meal non-feeding interval on the intake during the meal (pre-prandial correlation). Post-prandial correlations were calculated from linear regression of the intake during the meal on the duration of the interval until the start of the subsequent meal. These were calculated for pooled data and for individuals separately. It has been suggested (Savory, 1981; Machlis *et al.*, 1985; Dürst *et al.*, 1993) that correlations can be higher for specific periods in the day, therefore, correlations were calculated for all 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} meals in days with six meals because these were the most numerous in this data set.

5.4. Results

5.4.1. General

Table 5.2 shows that cows made a non-random diet choice resulting in, on average, proportionally 0.70 of their diet consisting of the H food. Estimation of individual meal criteria enabled the many visits (averaging approximately 30/cow/24h) to be clustered into meals resulting in an average of six meals per 24h. Cows demonstrated a diurnal pattern to their intake behaviour having a proportion of 0.59 of their visits and their meals during the day.

Table 5.2. *Average (± s.e.) of individual mean feeding behaviour variables.*

Feeding variables	
FMI (kg[b]/24h)	43.9 ± 0.74
DMI (kg[a] DM/24h)	20.6 ± 0.34
Diet choice (kg H/kg eaten[b])	0.70 ± 0.02
Number of visits/24h	31.9 ± 2.61
Meal criteria (minutes)	25.6 ± 1.47
Number of meals/24h	6.0 ± 0.21
Proportion of visits occurring in the day	0.59 ± 0.01
Proportion of meals occurring in the day	0.59 ± 0.01

[a]Dry matter.

[b]Fresh matter (as fed).

5.4.2. The probability of cows ending a visit or a meal

Figure 5.1a shows how the probability of cows ending a visit or meal changes with time since the start of a visit or a meal, for data pooled across cows and time of day. The meal ending probability increased with time since the start of the meal, however the rate of increase in ending probability was higher for meals of less than 30 minutes duration as compared to longer meals. In contrast, the change in probability with time since the start of the visit fluctuates around a mean of approximately 0.2, tending to decrease with visit durations of < 20 minutes then increase for longer visits. Figure 5.1b gives the ending probabilities for individuals with the highest (6.0 and 34.6 minutes) and lowest (2.9 and 16.6 minutes) average duration per visit or meal, respectively. The probability of ending a meal with time since the start of the meal was found to increase for individuals, however the rate of increase varied considerably depending on individual feeding patterns. Likewise, there was considerable individual variation in visit ending probabilities, however there was little systematic variation as visit duration increased. Figure 5.1c gives the ending probabilities for day and night separately. The meal ending probability was always higher during the night than the day, however both increased with time since the start of the meal. There was no difference between the pattern of ending probabilities for visits occurring during the day or the night. Figure 5.1d gives the ending probabilities of visits, or single food type meals. The ending probability was typically higher during the visits or meals consisting solely of L food, compared to H food.

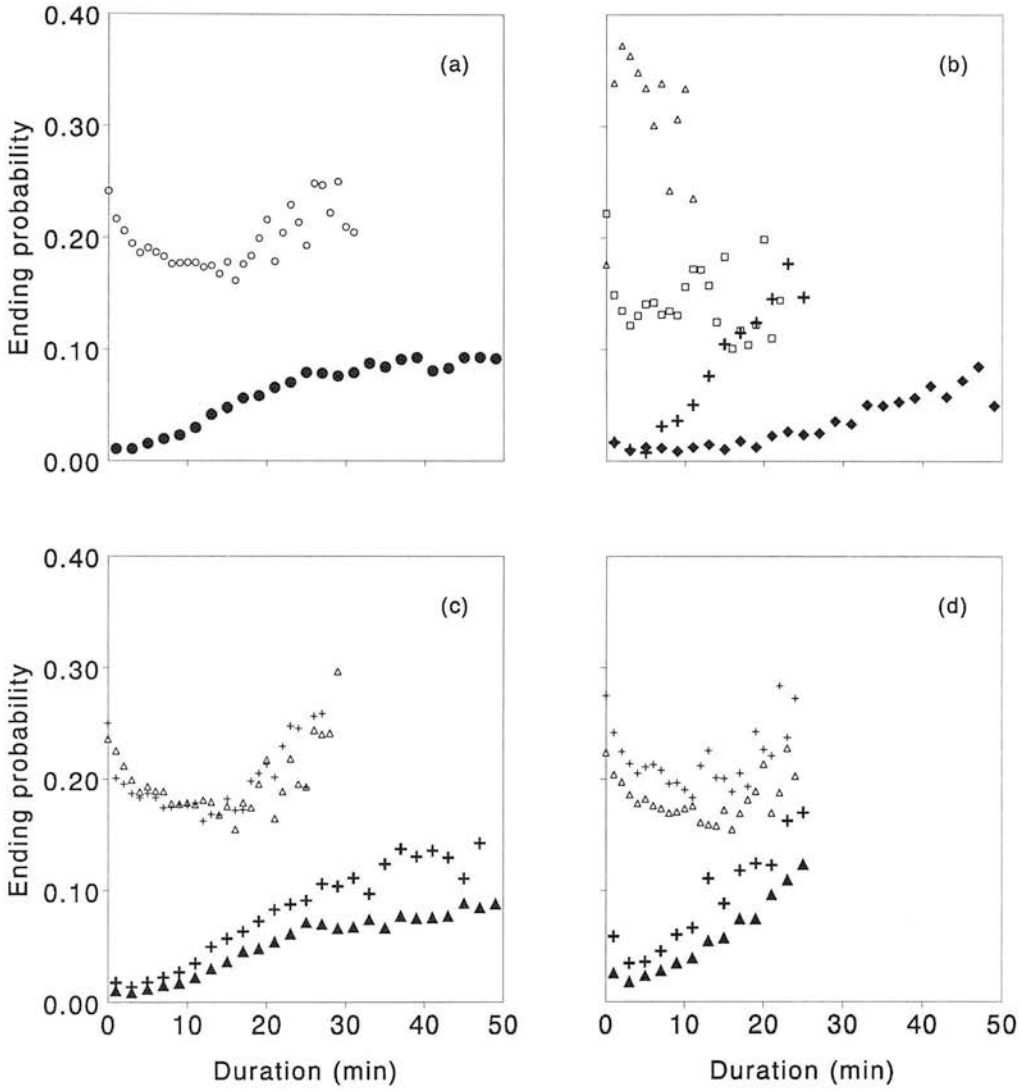

Figure 5.1. *The probability of a visit or a meal ending with time (1 minute) since the start of the visit or meal, respectively. Ending probabilities for visits (open circles) and meals (closed circles) from the pooled data (a). Ending probabilities for visits (open triangles and open squares) and meals (crosses and diamonds) from individuals with the highest and lowest average duration per visit or meal, respectively (b). Ending probabilities for visits (open triangles and thin crosses) and meals (closed triangles and thick crosses) for day and night, respectively (c). Ending probabilities for visits (open triangles and thin crosses) and meals (closed triangles and thick crosses) consisting of only high or low protein food, respectively (d).*

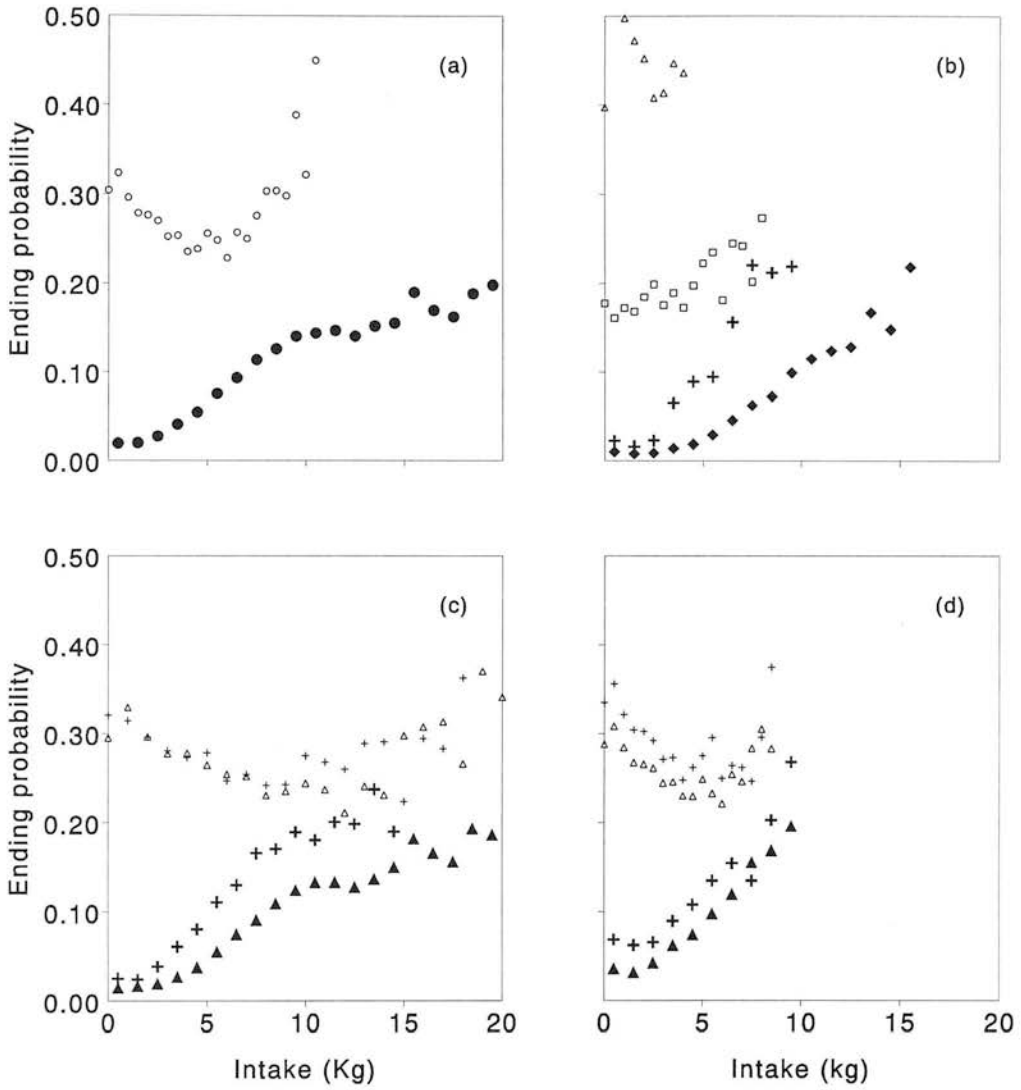

Figure 5.2. *The probability of a visit or a meal ending with quantity eaten (1kg) since the start of the visit or meal, respectively. Ending probabilities for visits (open circles) and meals (closed circles) from the pooled data (a). Ending probabilities for visits (open triangles and open squares) and meals (crosses and diamonds) from individuals with the highest and lowest average duration per visit or meal, respectively (b). Ending probabilities for visits (open triangles and thin crosses) and meals (closed triangles and thick crosses) for day and night, respectively (c). Ending probabilities for visits (open triangles and thin crosses) and meals (closed triangles and thick crosses) consisting of only high or low protein food, respectively (d).*

The change in ending probability with amount consumed since the start of the visit or meal is given in Figure 5.2. Figure 5.2a shows a higher rate of increase in meal ending probability for meals of less than 10kg, compared to larger meals. Figure 5.2b gives the changing ending probabilities for individuals with the highest (2.4 and 9.8kg) and lowest (0.8 and 6.1kg) average intake per visit or meal, respectively. There was considerable variation between individuals both for the visit and meal ending probabilities. All individuals displayed an increasing ending probability with intake during meals however there was no systematic change in visit ending probabilities. Figure 5.2c and 5.2d show that meal ending probabilities were higher during the night than the day and higher for meals consisting of L, compared to H food. Visit ending probabilities changed little with the amount consumed, and the pattern was not different between day and night or visits to H or L.

Figures 5.1a and 5.2a show that with data pooled across individuals and day and night, that the probability of ending a visit typically decreased then subsequently increased. To explore if this pattern was of biological relevance, or a product of pooling, the ending probabilities were averaged per minute (Figure 5.3a) or each 0.5kg eaten (Figure 5.3b) across cows. This shows that the ending probability did not systematically change with time, or intake. Indeed, they remained approximately constant when averaged across individuals.

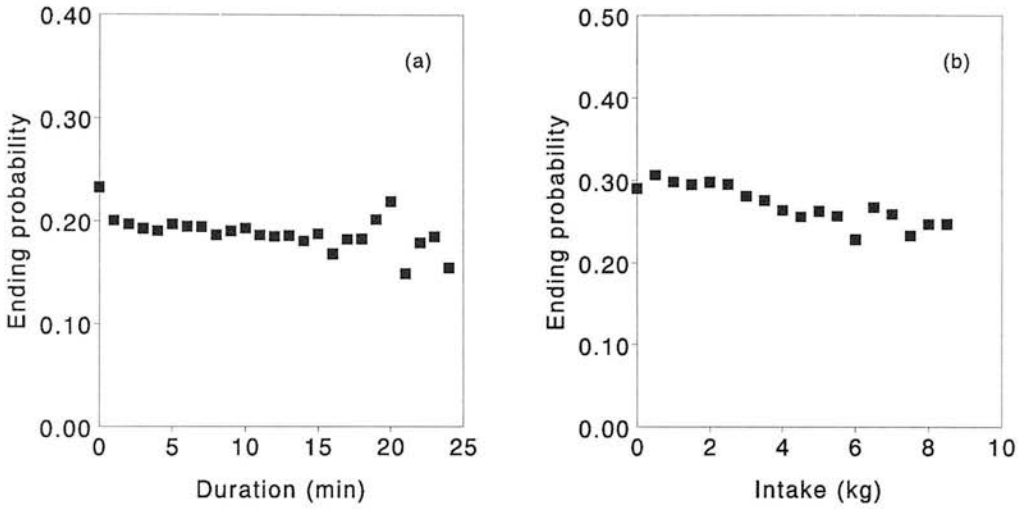

Figure 5.3. *The probability of ending a visit, averaged across individuals, with time (a) or intake (b) since the start of the visit.*

5.4.3. Diurnal feeding pattern analysis

Figure 5.4 gives the observed mean diurnal pattern in feeding behaviour for days in which between four and eight meals were consumed. The gap in the observations between approximately 08:00 and 09:30 of the cycle start times was a result of the cows having no access to feeders during this time, and as a consequence they could not initiate a feeding cycle. Figure 5.4a shows the average pattern of intake per hour of the feeding cycle and the fit of the sine wave model to the observations pooled across cows. The parameter values were: mean level (a_1) = 2.17kg, proportional amplitude (a_2) = 0.39 and shift in the sine wave (a_3) = 7.1h. The fit of the sine wave model was significant ($P < 0.001$) with an R^2 of 15% and a residual standard deviation of 1.39kg. Figure 5.4b gives the mean diurnal pattern in diet choice (kg H/kg intake) and the fit of the sine wave model. The parameter values were: mean

level (a_1) = 0.70, proportional amplitude (a_2) = 0.05 and shift in the sine wave (a_3) = 5.0h. The fit of the sine wave model was also significant ($P < 0.001$), however the R^2 was only 0.5% and the residual standard deviation was 0.32 kg H/ kg intake.

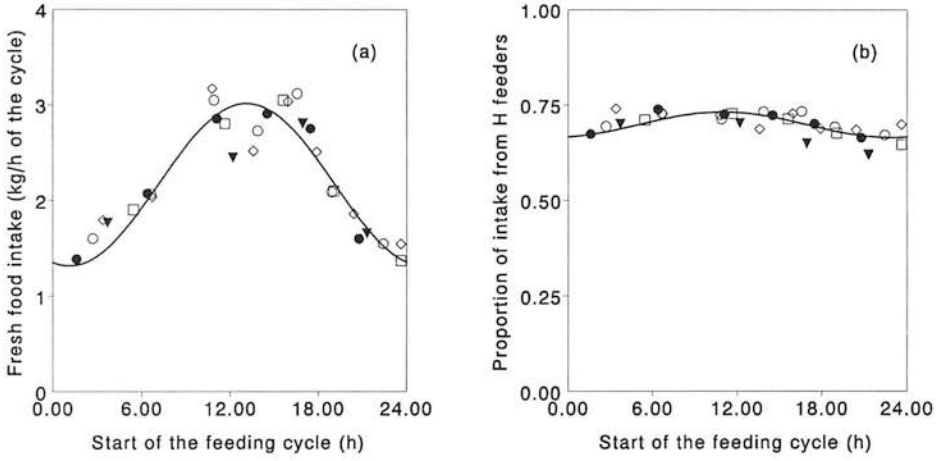

Figure 5.4. *The diurnal pattern of average intake (a) or average diet choice (kg H/Kg intake) (b) per hour of the feeding cycle (feeding cycle defined as the total duration of the meal plus the preceding non-feeding interval). Data shown are for cow days during which four (triangles), five (open squares), six (closed circles), seven (open circles) or eight (diamonds) meals were consumed. The line represents the fit of the sine wave model (see Table 5.3). 0.00 corresponds to midnight.*

Calculation of the sine wave amplitude as a proportion, i.e. the amplitude divided by the mean level of the sine wave, enables differences in the sine waves amplitude to be compared even when the scales of calculation are different. Therefore, intake which is expressed in kg (Figure 5.4a) and diet choice which is expressed in kg H/kg intake (Figure 5.4b) can be compared. This comparison shows that the proportional

amplitude of the sine wave model describing the diurnal pattern of intake is approximately eight times greater than that describing the diurnal pattern of diet choice.

The shift in the sine wave (a_3) indicates how the shape of the sine wave is related to the time of day. Therefore, this parameter enables comparison of how the sine wave changes in relation to time for both the intake (kg) and diet choice (kg H/kg intake) per hour of the feeding cycle. Comparison of Figures 5.4a and 5.4b shows that the shift parameters (a_3) of the sine wave are similar (7.1 and 5h, respectively). Therefore, analysis of the pooled data suggested that cows tended to eat a diet where their maximum intake per hour of the feeding cycle corresponds with the hours when they selected a slightly higher proportion of H food than average.

The models were also fitted to individual data (Table 5.3). This table shows that the average of the individually fitted model parameters were similar to those from models fitted to the pooled data, however the mean proportional amplitudes and correlation coefficients were higher in the data averaged across individuals as compared to the pooled data. The shift of the sine wave varied more between individuals when the model was fitted to the diurnal diet choice patterns of individuals (2.5 – 18.1h) than when fitted to individual's intake per hour of the cycle (5.3 – 8.4h). The fit of the sine wave model to individual intakes per hour of the feeding cycle was statistically significant for all cows ($P < 0.001$) and significant ($P < 0.05$) for 11 of the 16 cows when fitted to the pattern of diet choice.

Table 5.3. *Average (± s.e.) of individually estimated sine wave parameters and coefficients for the model*[a] $Y_i = a_1(1 + a_2SIN((2\pi/24)(t_i-a_3))) + e_i$ *fitted to intake and diet choice.*

	Fresh food intake (kg)	Diet choice (kg of H/kg intake)
Sine wave parameters		
a_1 (mean level)	2.15 ± 0.051	0.70 ± 0.013
a_2 (proportional amplitude)	0.41 ± 0.014	0.07 ± 0.008
a_3 (shift, h)	7.01 ± 0.245	6.27 ± 1.010
Correlation	0.42 ± 0.105	0.13 ± 0.072
R^2 (%)	17.7 ± 1.100	1.60 ± 0.522
Residual standard deviation	1.34 ± 0.061	0.31 ± 0.007

[a]In the model Y_i = intake per hour of the feeding cycle i (kg/h) or diet choice in feeding cycle i (kg H/kg of intake), a_1 = mean level of the sine wave, a_2 = proportional amplitude of the sine wave, a_3 = shift in the sine wave (h), t_1 = start time of the feeding cycle i (h), and e_i = error term.

5.4.4. Prandial correlations

Table 5.4 gives the pre- and post-prandial correlation coefficients. In the pooled data both pre- and post-prandial correlation coefficients were low, but statistically significant. The pre-prandial relationship had a very low R^2, accounting for less than 4% of the variation, and the post-prandial R^2 was even lower, accounting for less than 2% of the variation. Individual correlation coefficients included both negative and positive relationships, some of which were statistically significant. When these

were subsequently averaged they were lower than those calculated from the pooled data. Correlations calculated separately for each of the meals in six meal days were always positive and often statistically significant, but generally lower that those for the pooled data. There was no evidence of strong prandial correlations during particular parts of the day or night.

Table 5.4. *Pre and post-prandial correlation coefficients for pooled data, averaged across individual cows (± s.e) and averaged across the meal sequence number of all days with six meals (± s.e).*

	Pooled data	Mean of individuals ± s.e.	Mean of meal-sequence numbers ± s.e.
Pre-prandial			
Correlation	0.19	0.14 ± 0.024	0.12 ± 0.018
R^2 (%)	3.62	1.83 ± 0.056	1.45 ± 0.033
P	$P < 0.001$	$P < 0.001$	$P = 0.001$
Post-prandial			
Correlation	0.13	0.07 ± 0.022	0.12 ± 0.033
R^2 (%)	1.57	0.54 ± 0.048	1.48 ± 0.106
P	$P < 0.001$	$P = 0.004$	$P = 0.014$

5.5. Discussion

5.5.1. General

Previous work has suggested that the feeding behaviour of animals may be controlled by short-term depletion-repletion mechanisms (Le Magnen, 1985), i.e. control of short-term feeding behaviour is deterministic in nature. In contrast, the work presented in Chapter 4 suggested that animals are able to regulate their diet over longer timeframes and as a consequence of this have flexible short-term feeding behaviour which is better described as probabilistic in nature. Analysis of short-term feeding behaviour can then be used to address the issue of whether short-term feeding behaviour is better described as deterministic or probabilistic in nature. This may provide insight into the mechanisms animals use to regulate their food intake.

5.5.2. The probability of cows ending a visit or a meal

Short-term feeding behaviour is often interpreted in terms of the predictions from the satiety concept (Le Magnen, 1985; Simpson and Ludlow, 1986; Tolkamp *et al.*, 1998a). Indeed, this concept has been central to the analysis of hunger mechanisms conducted in Chapters 2 and 3 where its predictions have been shown to account for the short-term feeding behaviour of cows. This concept also predicts that as animals eat and become more satiated they will be less likely to continue feeding. In the present chapter this prediction is examined both in relation to intake and time since

the start of the meal. Therefore, in contrast to the work conducted in Chapters 2 and 3 where hunger mechanisms were investigated through calculation of meal starting probabilities, in this chapter satiety mechanisms have been explored through calculation of meal ending probabilities. The hypothesis that the probability of cows ending a meal would increase as they became more satiated, i.e. with time and/or intake since the start of the meal, was therefore investigated. Previous work (Tolkamp *et al.*, 2000) has suggested that the meal is a more biological unit of intake regulation than the visit. The ending probabilities of visits and meals were therefore contrasted to determine if differing patterns of ending probability were indeed evident.

The ending probabilities of visits were found not to systematically change for individual animals. An ending probability that neither increases or decreases indicates that the frequency distributions of visit duration and size can be described by a negative exponential distribution (Simpson and Ludlow, 1986). This implies that visits were terminated at random (Tolkamp *et al.*, 2000). In contrast to this, analyses showed that the probability of cows ending a meal increased systematically. This was the case irrespective of whether ending probabilities were assessed in terms of duration or intake since the start of the meal. Therefore, the principle of satiety was applicable to feeding whether this was represented by the time spent at the feeder or the actual amount of food eaten during meals, but not during visits.

In light of the results presented in Chapter 3, where pooling was found to affect the interpretation of feeding behaviour, the consequences of pooling for understanding

visit ending probabilities was also investigated. Pooling of data prior to calculation of visit ending probabilities was found to result in pooled ending probabilities that appeared to decrease then increase, despite the fact that there was no systematic change in individual visit ending probabilities. This could be a result of some cows typically having very high, or very low, visit ending probabilities. Pooling such data would result in pooled probabilities that were largely determined by some cows at short durations (or low intakes) and other cows at long durations (or high intakes). Averaging of individually calculated probabilities (Figure 5.3) showed that this apparent pattern of visit ending probabilities was an artefact caused by inappropriate pooling.

The calculation of meal ending probabilities showed, in agreement with analysis presented within Chapter 3, that individual animals can have widely differing feeding behaviours. Ending probabilities were also seen to be systematically higher during the night than the day. This reflected diurnal differences in feeding behaviour with, on average, larger meals occurring during the day than the night. Analysis within this chapter showed that pooling of data, across day and night or individuals, typically resulted in a decreased rate of ending probability increase, at longer interval lengths. Therefore meal-ending probabilities seemed to increase more slowly in the pooled as compared to the un-pooled data. This is in agreement with the findings presented in Chapter 3 where it was shown that inappropriate pooling could result in misinterpretation of feeding behaviour leading to a poorer understanding of how intake is regulated.

Calculation of ending probabilities for meals consisting of only one food type could give unique insight into how satiating individual foods are. This can show, from the analysis of animal behaviour, how animals interact with different foods. Therefore, if feeding patterns are linked to the food type being consumed then this will be reflected in the calculated probability of ending a meal. Indeed, previous work by Tolkamp *et al.* (2000) found that cows offered a low protein food had a consistently higher probability of ending a meal compared to different cows that were offered a high protein food. This suggested that the low protein food was more satiating. In contrast to the study of Tolkamp *et al.* (2000), work within this chapter compared the probability of cows ending a meal when offered a choice of foods. This work showed that during meals consisting of visits solely to the H or the L feeder then cows had a consistently higher probability of ending a meal when eating the L, as compared to the H, food. Within Chapter 4 it was shown that choice fed cows had, on average, greater visit durations when feeding at H feeders as compared to L feeders. Therefore, greater average visit duration could be expected to result in a longer meal and lower ending probability at H, as compared to L, feeders. The fact that cows make a non-random diet choice however suggests that here must be some benefit to eating the more satiating L food. The mechanisms behind this diet choice process remain unclear.

This analysis has shown, in agreement with the first hypothesis presented, that the probability of ending meals increases both with time and intake since the start of the meal. This was not the case when ending probabilities were assessed in terms of visits. Therefore, the probability of ending a meal is well described by the predictions

of the satiety concept. This work therefore provides further evidence, in agreement with the findings of Chapters 2, 3 and 4, that the feeding behaviour of cows is best described as probabilistic in nature.

5.5.3. Diurnal pattern analysis

In previous studies it has been assumed that animals regulate their diet in a short-term deterministic way (Le Magnen, 1985). If this is the case then it should be evident from the analysis of short-term feeding behaviour whether feeding behaviour is regulated, and therefore predictable, in the short-term. Great flexibility of short-term feeding behaviour would not be expected if animals regulate their diet in the short-term, as such a strategy is more indicative of diet regulation over a longer time frame. One approach to studying diet regulation through short-term feeding behaviour analysis is to assess any diurnal patterns in intake and diet composition. If animals regulate a diet in the short-term than it may be expected that any diurnal pattern in the diet consumed is repeatable and does not display great flexibility depending on the feeding environment.

Tolkamp *et al.* (2002) showed that cows fed a single food, rather than a choice, had an average intake per hour of the feeding cycle that fluctuated through the day and night following a sine wave pattern. This resulted in, on average, greater intakes for feeding cycles that started around midday as compared to midnight. Analysis within this chapter, using choice fed cows, confirmed the findings of Tolkamp *et al.* (2002), as all individuals had a statistically significant sine wave pattern in their average

intake per hour of the feeding cycle. Indeed, the diurnal feeding patterns of individuals were remarkable similar, as demonstrated by the low standard s.e.'s associated with the group average sine wave parameters (Table 5.3). This suggests that higher intake for cycles starting in the day, as compared to the night, was typical for cows. However, it must be noted that there was considerable variation about this pattern with the R^2 of the fit averaging 18% and a residual standard deviation of 1.35kg. This indicates that despite the strong diurnal pattern in intake there is still considerable variation about the sine wave pattern of intake. Therefore, although greater intakes during the day, as compared to the night, are thought to be favoured behaviour (Forbes, 1995a), it is clear that the feeding behaviour of cows varies greatly about this pattern. This analysis has therefore not provided evidence in favour of short-term diet regulation.

Analysis of the diurnal pattern of diet choice, for data pooled across cows, showed the proportion of H food selected to be marginally higher during the day than the night. However, the proportional amplitude of the sine wave was very small (0.05) indicating that the average diet selected by the cows was very similar in all hours of the day and night. However, between individuals there was large variation in the sine wave shift parameter, i.e. in the time of day that they ate a diet with the greatest proportion of the H food. Indeed the difference between the lowest and highest value of this parameter was greater than 12 h. Therefore, at a given time some cows were beginning cycles during which they consumed meals with, on average, a high proportion of H food whilst at the same time other cows were beginning cycles where they ate meals with relatively little H food. Across individuals this model

accounted for just 1.6% of the variation in the data, giving a large average residual standard deviation of 0.31 kg H/kg intake. This shows that there is little, if any, diurnal pattern in individual diet choice, indeed the variation about the observed sine waves was relatively great compared to the variation that the sine wave pattern accounted for. Analysis of pooled data therefore suggested a stronger diurnal pattern in the proportion of H food in the diet than was actually selected by the individual cows. In agreement with the conclusions of Chapter 4, this analysis provides no evidence in favour of animals regulating the composition of their diet in the short-term.

Previously, Tolkamp and Kyriazakis (1997) have shown that cows, offered a choice between a high and a low protein food, select a diet which is lower in protein during the day, when compared to the night. This led to the hypothesis that a similar diurnal pattern of diet choice would be demonstrated within the experiment analysed in this chapter. However, the finding, that diurnal variation in diet choice accounted for very little of the variation in observed feeding behaviour and that, if anything, that there was a higher diet choice during the day must lead to the rejection of this hypothesis. The reason for the different feeding behaviour shown between the cows in the two experiments was probably related to the fact that the cow pressure per feeder was higher at the feeders supplying high, compared to low, protein food in the study reported by Tolkamp and Kyriazakis (1997). Cow pressure has been shown to greatly affect feeding behaviour of cows (Elizalde, 1993; Chapter 4) and in the experiment of Tolkamp and Kyriazakis (1997) likely accounted for the cows selecting a lower protein diet when feeders were busy (during the day). At these times subordinate

cows would have had less access to feeders supplying the higher protein food and as a consequence may have eaten the lower protein food. This would likely have led to compensation at times when the feeders were less busy (during the night). Hence a diurnal pattern of diet choice was observed by Tolkamp and Kyriazakis (1997), with less of the high protein food eaten during the day. In contrast to the experiment reported by Tolkamp and Kyriazakis (1997), the cow pressure per feeder was the same for all feeders in the experiment reported within this chapter. Therefore, these results suggest that if cows are able to access feeders supplying the two food types with equal ease (i.e. not prevented by unequal cow pressure) then there is no biologically relevant diurnal pattern in diet composition. Indeed, the evidence suggests that animals have very flexible feeding behaviour that is responsive to the feeding environment rather than regulated in the short-term.

5.5.4. Prandial correlations

The presence of prandial correlations has historically been interpreted as strong evidence in favour of short-term mechanisms controlling food intake (Le Magnen, 1985). Indeed, pre-prandial correlations have been interpreted to indicate the role of satiety mechanisms because the quantity of food eaten in a meal is linked to how satiated that animals becomes, which in turn is related to the time since the last meal. Conversely, post-prandial correlations are thought to indicate the role of hunger mechanisms because the amount of food consumed in a meal will influence the time until the animal becomes hungry again and begins to eat (Dado and Allen, 1994; Savory, 1999). Therefore, prandial correlations are thought to provide evidence for

short-term depletion-repletion feeding strategies where each meal begins when the store of fuel from the previous meal is exhausted (Le Magnen and Devos, 1980).

Within this study, and in agreement with previous work (Metz, 1975; Mayes and Duncan, 1986; Dado and Allen, 1994; Tolkamp *et al.*, 2002), pre-prandial correlation coefficients were found to be greater than post-prandial coefficients. However, in agreement with many previous studies (e.g. Savory, 1981; Bigelow and Houpt, 1988), the R^2 of the correlations were always very low. This indicates that the length of the pre-meal non-feeding interval had almost no effect on the size of a meal and that intake during a meal had even less of an effect on the length of the subsequent non-feeding interval.

Dürst *et al.* (1993) suggested that stronger prandial correlations can be found at certain times of the day and that these can be obscured by calculating correlations over the whole day. This finding was linked to the calculation of correlations with data pooled from periods with many meals (the day) and periods with few meals (the night). Calculation of prandial correlations for each of the six meals separately showed that the correlations were mostly positive and significant, however the strength of the relationships were generally not stronger than those in the pooled data. This suggests that, at least for these cows, prandial correlations are very weak throughout the day and night.

Individual coefficients were generally even lower than those from pooled data, which agrees with findings of Tolkamp *et al.* (2002). The analysis presented within this

chapter shows that pooling information from individuals, some of which show negative or non-significant correlations, can result in positive correlations that are statistically significant. This agrees with previous findings that prandial correlations can be artificially inflated by inappropriate pooling (Panksepp, 1973; De Castro, 1975; Bigelow and Houpt, 1988; Demaria-Pesce and Nicolaidis, 1998). This can be a consequence of pooling data across individuals, some of which have many meals per 24h and consequently low intakes per meal and short between meal intervals, and others with few meals per 24h, and on average large meals and long intervals. Despite this, even in pooled data the R^2's remained very low.

The analysis conducted within this chapter, of a data set which is considerably larger than those typically used to study short-term feeding behaviour, has provided evidence that contradicts the conclusions of Le Magnen and Devos (1980) that prandial correlations provide evidence of short-term diet regulation. Therefore, the hypothesis that animals regulate their diet in the short-term and that this would be indicated by the presence of strong prandial correlations must be questioned. Indeed, even when prandial correlations are calculated on data which has been pooled, with the result that prandial correlations are artificially inflated, this analysis refutes the idea that short-term feeding behaviour is structured such that animals can maintain a regular diet in the short-term.

5.5.5. Conclusions

The analysis of short-term feeding behaviour can provide evidence of how animals structure their feeding behaviour, which can then give an insight into how food intake is regulated. If regulation occurs in the short-term, as a result of depletion-repletion, i.e. deterministic, mechanisms of diet regulation then this should be evident from such analysis. Therefore, the probability of ending a meal should not be well described as probabilistic. However, in contrast to this expectation the feeding behaviour of cows was well described as probabilistic. This was in agreement with the analysis of prandial correlations and diurnal patterns which provided no evidence in support of short-term depletion-repletion mechanisms but instead suggested that animals were very flexible in their short-term feeding behaviour. In conclusion, the work presented in this chapter has provided no evidence that feeding behaviour is regulated within the short-term. Indeed, this work suggests that the time frame of diet regulation must be greater than the short-term. Flexible short-term regulation would enable animals to better respond to changes in the feeding environment and therefore adapt short-term feeding behaviour whilst still maintaining an appropriate diet in the medium-term. Analyses such as these can then provide invaluable information with which to assess ideas and theories of how animals regulate their diet.

CHAPTER SIX

Short-term feeding behaviour and its implications for understanding food intake regulation

6.1. Introduction

Within the animal and ecological sciences there has long been interest in predicting both the diet that animals select and the quantity of that diet that they will consume (Forbes, 1995a). One approach to this problem has been to study the mechanisms that animals use to regulate their intake and diet composition as a better understanding of this may result in improved intake and diet composition prediction models. Despite this, after many years of scientific endeavour, there is still no consensus on how animals regulate their food intake (Weston, 1996).

At least formally, the long-term diet that animals consume must be the sum of the diet selected in the short-term. Therefore, further insight into feed intake and diet composition regulation mechanisms may be gained by studying the relationship between the diet that animals consume and their short-term feeding behaviour (Forbes, 1980; Gill and Romney, 1994). Analysis of short-term feeding behaviour can then be used to address questions such as whether feeding behaviour is controlled by short-term deterministic feed back mechanisms or, alternatively, whether animals aim to achieve a certain diet in the medium-term and consequently have flexible short-term feeding behaviour which is better described as probabilistic in nature. In essence this is a question of cause and effect between the diet selected in the short-term and the intake and diet composition achieved in the longer-term.

Before such analysis can be undertaken it is important to ensure that the methodology of short-term feeding behaviour analysis is sufficiently robust to enable

sound conclusions to be drawn about the role of short-term feeding behaviour in the regulation of intake and diet composition. Therefore, within this chapter methodological developments made within the thesis will be assessed and linked to previous attempts to understand short-term feeding behaviour. Findings from the analysis of short-term feeding behaviour will be used to further develop ideas of how short, medium and long-term intake are linked and subsequently to assess the direction of cause and effect between these time scales of intake regulation. Conclusions from this will be used to assess the predictions from previously published theories of intake regulation, in terms of short or medium-term diet regulation. Theories most in agreement with the evidence will be highlighted. This information will be used to provide direction for future work that aims to predict food intake and diet composition through a better understanding of the underlying biological regulation mechanisms.

6.2. Methodological issues

6.2.1. Intake of a single food

In order to gain a better knowledge of intake regulation through analysis of short-term feeding behaviour it is important to understand the structure of feeding behaviour. Therefore the first question must be, what are the units in which animals organise their feeding behaviour? The shortest unit of feeding behaviour that can be recorded depends on the methodology used and the animal being studied, e.g. licks,

pecks, bites or visits to the feeder (e.g., Sanderson and Vanderweele, 1975; Parsons *et al.*, 1994; Rushing *et al.*, 1997; Shipley *et al.*, 1999; Slater, 1974; Tolkamp *et al.*, 2000; Whittemore *et al.*, 2002). The issue of whether these units are the most relevant for analysis of short-term feeding behaviour must therefore be addressed. The approach taken within this thesis has therefore been to assess the mathematical properties of short-term feeding behaviour with the aim of understanding how animals organise their behaviour and thus regulate their intake.

6.2.1.1. The negative exponential distribution for describing short-term feeding behaviour

For housed cows, on which this thesis has centred, the shortest unit in which feeding behaviour could be recorded was a visit to the feeder. Previous attempts to assess short-term feeding behaviour, in terms of visits, have often assumed that visit characteristics can be well described by a negative exponential distribution (Slater, 1974; Slater and Lester, 1982; Sibly *et al.*, 1990; Berdoy, 1993). This implies that the feeding behaviour of animals is not ordered (Slater, 1974; Sibly *et al.*, 1990; Tolkamp *et al.*, 1998a). However, on further inspection of the mathematical properties of these aspects of feeding behaviour this was found not to be entirely the case (Tolkamp *et al.*, 1998a; Tolkamp and Kyriazakis, 1999a; Tolkamp and Kyriazakis, 1999b; Chapter 2).

If the intervals between visits were well described by a negative exponential, i.e. were random, then one would expect there to be many very short intervals. However,

when exploring the frequency distribution of intervals there was clearly a lack of very short interval lengths. This can be attributed to the time scale of measurement being short in comparison to the physical limitations of the behaviour being performed (Sibly *et al.*, 1990). Therefore, although animals could stop feeding at one feeder and, at least in theory, be recorded as beginning feeding at another feeder within one second this was physically unlikely to occur. Therefore, instead of recording many very short intervals, i.e. a few seconds, there were fewer such intervals recorded than predicted by the negative exponential. The influence of physical limitations affecting feeding behaviour was shown within Chapter 2 where the intervals during which cows visited the drinker were given. This showed that the intervals during which cows visited the drinking trough were approximately two minutes or greater indicating that this was the minimum time for cows to travel to the drinker, consume water and return to the feeder. Therefore, a negative exponential distribution cannot describe such behaviour indicating that visits are not started at random.

In contrast to the failure of negative exponentials to fit the distributions of intervals between visits at their lower ends, i.e. shortest durations, this distribution fitted the visit durations and intakes per visit very well. This implies that visits were terminated at random. This was indeed found to be the case within Chapter 5 where it was shown, in agreement with Tolkamp *et al.* (2000), that the probability of visits ending was not related to the duration or the amount consumed during the visits. Therefore, visits were terminated at random.

6.2.1.2. *Clustering of short-term feeding behaviour*

Upon further examination of short-term feeding behaviour it is clear that visits to the feeder (or other measures of short-term feeding behaviour) are generally clustered into higher order bouts of feeding behaviour, i.e. meals (Slater and Lester, 1982; Forbes, 1985; Adenuga *et al.*, 1991; Shorposner *et al.*, 1994; Tolkamp *et al.*, 1998a; Collier *et al.*, 1999). The reason that animals consume food in meals has been open to some debate, indeed various ideas have been proposed. These include the idea that animals may eat until some short-term feed back mechanism instructs that animal to cease feeding. The feed back may be a result of the animal feeling full or alternatively the animal sensing that it has replenished some nutritional deficit (see below for further discussion on the theories of food intake regulation). Common across most theories of intake regulation is the concept of satiety. This has been defined by Le Magnen (1985) as a passive state of no hunger. Satiety is the state during which, from the end of one meal to the beginning of the next meal, an animal does not eat (Le Magnen, 1985). There has been considerable debate in the literature about the mechanisms which animals use to regulate their intake (e.g., Booth, 1978; Le Magnen, 1985; Forbes, 1988; Mertens, 1994; Forbes, 1995a; Forbes, 1999; Pittroff and Kothmann, 1999; Ellis *et al.*, 2000; Forbes, 2000). This demonstrates that the precise physiological basis of satiety is as yet unclear (Forbes, 1995a). Despite this, there is a general consensus of opinion that animals terminate a meal when satiated and subsequently begin another meal when the satiating effects of the previous meal have subsided (Metz, 1975; Le Magnen, 1985; Forbes, 1995a; Simpson and Ludlow, 1986). Therefore, analysis of feeding behaviour in terms of

meals can provide a common currency for comparison of behaviour in situations where the shortest unit of behaviour recorded differs. Additionally, it is possible that meals are more biologically relevant than visits and potentially that these are the units over which feeding behaviour is regulated. Indeed, previous work has shown that, whereas feeding events are greatly affected by random processes, e.g. interactions between animals and the environment, meals are, in contrast, a more biologically relevant unit (Tolkamp *et al.*, 2000).

6.2.1.3. Clustering of short-term feeding behaviour using a meal criterion

If analysis of short-term feeding behaviour in terms of meals is a useful approach, then a reliable way of clustering observed feeding behaviour into meals is needed (Sibly *et al.*, 1990; Berdoy, 1993; Tolkamp and Kyriazakis, 1999b). Historically, this has been approached in many different ways. Initially, an observation based approach was used whereby the start and end of meals were defined whilst observing the animal. This approach has a number of problems including being very subjective and limited to the relatively small data sets which can be collected by an individual watching animals feed (see Forbes, 1995a for further discussion). Therefore, a rule (or criterion) based approach to clustering feeding events into meals was required. This led to the development of a meal criterion, which is the longest non-feeding interval between visits to the feeder that are considered to be part of separate meals. Initially, this approach was used to define a meal criterion before analyses of feeding behaviour data, e.g. Simpson (1982), where a meal criterion of four minutes was

assigned. Although the assigning of a meal criterion *a priori* removes the subjectiveness from deciding when individual meals start and finish this must been seen as arbitrary and is likely to lead to incorrect clustering of feeding events into meals. On this basis it should be rejected.

Mathematical approaches to the estimation of meal criterion have also been proposed, such that the meal criterion is chosen after analysis of the feeding behaviour data. These include using frequency analysis (Forbes *et al.*, 1986; Bermudez *et al.*, 1989), log-survivorship analysis (Slater and Lester, 1982) and log-frequency analysis (Sibly *et al.*, 1990; Berdoy, 1993). The first of these approaches estimates the meal criterion from fitting two straight lines to the frequency distribution of interval lengths. The intersection of these lines is defined as the meal criterion. The latter two methods essentially consist of fitting negative exponential models to the distribution of non-feeding intervals between meals. From these distributions a meal criterion is estimated. However, as discussed earlier the negative exponential distribution is not suitable for describing the shortest non-feeding intervals. Additionally, an implicit assumption of the negative exponential distribution is that the probability of animals starting a meal is assumed to remain constant, irrespective of the time since the last meal (Tolkamp and Kyriazakis, 1999a). Therefore, animals are assumed to be no more likely to begin a meal after not eating for a few hours than after a few minutes. This clearly contradicts the biological expectations of the satiety concept (Le Magnen, 1985) which predicts that as animals get hungrier they are more likely to begin feeding.

6.2.1.4. *Clustering of short-term feeding behaviour using a biologically based meal criterion*

In response to finding that the biological basis of previous meal criterion models, based on negative exponentials, were flawed Tolkamp and colleagues developed another approach to the estimation of a meal criterion. This involved fitting two (Tolkamp *et al.*, 1998a) or three-population (Tolkamp and Kyriazakis, 1999b) Gaussian models to the intervals between feeding events. The predictions of these models were in far better agreement with the observations than previously published models. However, further analysis of these Gaussian models showed they predicted that the probability of a meal starting after a non-feeding interval first increased, in line with the biological expectations of the satiety concept, but subsequently decreased (Tolkamp and Kyriazakis, 1999a; Chapter 2). Therefore, the predictions were still at odds with biological expectations, at least at longer interval lengths (Tolkamp and Kyriazakis, 1999a; Chapter 2).

In conclusion, the effectiveness of exploring short-term feeding behaviour, in order to better understand how animals regulate their diet, is very dependent on appropriate analysis being undertaken. Previous models, which have attempted to estimate meal criteria have all suffered from an incompatibility of the biological expectations and the predictions from the mathematical distributions used to describe the intervals between meals. Therefore, the mixed distribution model which was proposed in Chapter 2 is a strong contribution as it is both statistically and biologically superior to previously proposed models, being in excellent agreement with observations and

with the predictions of the satiety concept (see Chapters 2, 3, and 5). Additionally, the work conducted within Chapter 3, where novel simulation models were constructed has enabled a systematic investigation of when the variation within the data set was sufficiently great that pooling could be problematic. This has highlighted further potential problems for the analysis of short-term feeding behaviour. This work has therefore contributed a reliable methodology, which has been used within this thesis, and elsewhere (Tolkamp *et al.*, 2002; Whittemore *et al.*, 2002), to explore the question of how animals organise their feeding behaviour.

6.2.2. Intake when offered a choice of foods

In conditions typically found in nature, and when domesticated animals are offered a choice of foods, animals have to make decisions which are similar to those made when offered just one food, i.e. that of how much to eat of the given foods. Therefore, there has been considerable interest in the question of how animals regulate a diet that consists of a choice between foods (Galef, 1991; Kyriazakis and Emmans, 1991; Kyriazakis, 1997; Provenza *et al.*, 1998; Scott and Provenza, 1999). Analysis of such situations could also offer insight into the mechanisms animals use to regulate their diet. Indeed a better understanding of the time frame of diet composition regulation may prove insightful, yet despite this there has been very little work to date on this subject.

The majority of previous work in this area has focused on exploring how animals tolerate a forced deviation from their usual feeding behaviour, e.g. by offering two

foods, but on alternative days rather than simultaneously. Such work generally demonstrates that animals are able to tolerate a forced deviation from their usual diet composition without detrimental effects on medium-term consumption (Kyriazakis and Emmans, 1990; Forbes and Shariatmadari, 1996, Ludden *et al.*, 2002). Within Chapter 4 this approach was extended to examine how animals organise their short-term feeding behaviour when offered a free choice of two foods. This work relied on the development of a new analysis technique to determine if animals attempted to consume a consistent ratio of the two foods within the time frame of meals. No evidence was found for this occurring, indeed the behaviour of cows was well described as probabilistic in nature.

The problem for future work is therefore to identify the time frame over which animals maintain a consistent diet. One approach to this may be to extend the analysis presented within Chapter 4 to determine if the composition of a meal is affected by the composition of the previously eaten meal. If such a relationship exists it would be expected that there would be a negative correlation between the composition of a meal and that of the following meal, i.e. if a meal was composed of visits to the feeder supplying the H food then it would be followed by a meal consisting of mostly visits to the L feeder. To date attempts to explore this have however been unsuccessful. Indeed, such problems as mild fluctuations in the composition of the foods can cause slight changes in the overall composition of the diet selected, which results in flawed statistics. Problems such as this can be minimised by careful control of the experimental diets. However, when feeding forage based diets, e.g. silage, then slight fluctuations in the quality of the diets can

be difficult to control. Therefore, if for example the long-term trend for the diet chosen consists of 40% food A and 60% food B but due to some slight change in one of the foods there are a few days where animals consume 60% A and 40% B then this can greatly influence any statistical analysis. Indeed, if there is no relationship between the composition of a meal and the following meal but one analysed the two periods (the 40% food A and 60% food A periods) together then instead of finding that there is no relationship between meals in fact a positive correlation will be found. This is statistically correct but biologically flawed as there was no relationship between the composition of one meal and the next. Similarly, if there was in fact a negative relationship between the composition in one meal and the composition of the following meal but again there are slight shifts in food quality over time then a genuine negative correlation can result in no correlation, or even a positive correlation, if the data set is analysed as a whole. Therefore, unless analysing very carefully controlled experiments over very short time periods then it is unlikely that such correlation analysis will be fruitful.

Future work on this subject needs to focus on identifying patterns in diet composition which may enable a better understanding of what animals are attempting to achieve with the diet they select. This work may be better to focus on relative diet composition, i.e. if the current meal contains a greater or lesser proportion of one of the foods offered as compared to the previous meal, rather than examining the actual diet composition. Such an approach may enable some of the statistical pitfalls caused by slight variations in the diet quality to be avoided. Indeed, approaches such as detrended fluctuation analysis (Peng *et al.*, 2000), where diet composition could be

converted to a binary code according to relative diet composition may enable long-term patterns to be identified. Approaches such as this must be carefully explored to avoid erroneous conclusions arising from statistical bias.

6.3. The nature of the relationship between short-term feeding behaviour and long-term intake

When animals are fed a restricted diet then their long-term consumption is necessarily the result of their intake in the short-term. Therefore, long-term intake can be calculated from the diet consumed in the short-term. Similarly, when animals are offer *ad libitum* food supply but the number of cows per feeding space, i.e. the cow pressure, is high then limitations to intake can occur within the short-term. Such a situation was well documented by Elizalde (1993). In this experiment the cow pressure was increased from 1 to 9 cows per feeder. With cow pressure of 7 cows per feeder or below intake were seen to be unaffected by the number of cows per feeder, however short-term feeding behaviour was greatly affected with higher intake rates as competition increased. With more than 7 cows per feeder a physical limitation to feed access was encountered and intake levels could not be maintained. In such an extreme situation analysis of short-term feeding behaviour could result in accurate prediction of the intake levels that cows would achieve. Therefore, in situations where animals are unable to adapt behaviour to maintain intake levels then the direction of cause and effect is clearly that limitations in the short-term results in the long-term intake level.

In contrast to the above examples, when animals are given free access to foods then they display very flexible short-term feeding behaviour without compromising their long-term level of intake. This was clearly shown by Friggens *et al.* (1998) when the intake and short-term feeding behaviour of cows was measured. They showed that whereas some cows had many visits to the feeder and ate little per visit others had few visits to the feeder but ate more per visit such that both resulted in the same daily intake. This shows that animals can achieve the same long-term level of intake with many different combinations of short-term feeding behaviour. The wide variety of patterns of short-term feeding behaviour which animals display does not suggest that the animals are attempting to maintain a particular species-specific short-term feeding pattern to ensure a long-term intake goal is achieved.

If animals have short-term deterministic regulation of the diets they consume, i.e. if the arrow of cause and effect is regulation in the short-term resulting in the longer-term diet then one can expect the short-term feeding behaviour of animals to follow a characteristic pattern. This would result in strong relationships between both the intake in a meal and the length of the preceding non-feeding interval and between meal intake and the interval after the meal. Indeed, historically, prandial correlations have been presented as evidence of short-term regulation of feeding behaviour (Le Magnen and Devos, 1980; Schilstra, 1981).

To address this, analysis was undertaken in Chapter 5 to investigate the significance of prandial correlations by analysis of a data set that is much larger than those

typically used in studies of short-term feeding behaviour. This work showed that although prandial correlations could be statistically significant, and were inflated by pooling, they are of little biological relevance and account for almost none of the variation in the data. Therefore, the analysis within this thesis casts serious doubts on previous work suggesting that the presence of prandial correlations constitute evidence in favour of short-term regulation of intake. This was in agreement with the findings of Tolkamp *et al.* (2002) where neither in animals fed diets with a high proportion of silage or a high proportion of concentrate did they have strong prandial correlation. Weak correlations suggest that animals are not simply acting on short-term depletion – repletion mechanisms, where food is consumed until a short-term nutritional deficit has been resolved, as have been suggested (Le Magnen, 1985).

Short-term diet regulation could also be important for maintaining the best rumen environment by balancing nutrient supply and thus maximising the efficiency of food use (Sinclair *et al.*, 1995; Kim *et al.*, 1999a; Witt *et al.*, 1999a). It was therefore predicted within Chapter 4 that when animals were offered a choice of foods they would compose their meals in such a way as to ensure an optimum diet in the short-term. No evidence was found for animals regulating their diet within the short-term time frame of meals by attempting to visit the feeders supplying the different foods in the same ratio as the long-term average diet composition. Neither was there evidence of animals adjusting their intake per visit depending on the ratio of visits in the meal to result in meals with diet composition that were similar to the long-term average. Therefore, this work provided no evidence to support the idea that animals would attempt to maintain a consistent diet in the short-term, thereby ensuring a

regular diet in the longer-term. Indeed, cows were shown to compose meals in a probabilistic way that resulted in widely differing meal compositions in the short-term but relatively consistent weekly diet choice.

If animals do not regulate their intake within the short-term timeframe of meals then they may attempt to consume a constant daily diet. Indeed, Parsons *et al.* (1994) showed that when sheep were offered a choice of grass and clover they had a diurnal pattern in diet composition such that the preference for clover decreased with time during the day reaching a nadir in early evening. This diurnal preference for clover was attributed to the advantages of eating a diet consisting of a greater proportion of grass prior to the long night-time rumination period. The slower passage rate of grass being more suited to rumination over night, thus allowing quickly digested clover to be consumed during the active foraging period, i.e. in the day. In contrast, the analysis presented within Chapter 5 showed that the pattern of diet choice did not vary diurnally. Therefore, the apparent advantages of a diurnally varying diet, as shown within Parsons *et al.* (1994), seem less important for cows offered the H and L foods.

Analysis within Chapter 5 also showed that the probability of cows ending a meal increased with the food eaten during the meal rather than being relatively constant until a characteristic amount of food was eaten then becoming very high. Such a pattern of ending probabilities would suggest that the mechanisms controlling the end of each meal was a short-term signal dependent on the intake during the meal. Therefore, no evidence was found to support this from analysis of meal ending

probabilities. This is supported by the analysis of meal starting probabilities within Chapters 2 and 3. In this work the probability of starting a meal was shown to increase with time since the last meal. Probabilities did not show evidence of meals being controlled in the short-term, e.g. always beginning after a certain amount of time since the last meal. Therefore, it seems more appropriate to describe the short-term feeding behaviour of animals as probabilistic, rather that deterministic, in nature.

Additional evidence regarding the likely timeframe of intake regulation was provided by Allcroft *et al.* (1999). In this work the patterns in food consumption were assessed in terms of intake for animals offered *ad libitum* access to a single food. Despite detailed mathematical analysis this work failed to identify a short-term time period over which intake was regulated, indeed this work pointed to regulation over a time period of a day or greater.

The evidence presented above strongly suggests that animals do not attempt to maintain a consistent diet in the short-term. Therefore, the arrow of cause and effect between short-term feeding behaviour and long-term intake must surely be that intake is regulated over a medium-term time scale such that behaviour in the short-term can be flexible. This is despite the potential benefits of short-term diet regulation, which would ensure the correct nutrients were consumed in the short-term and consequently an optimum diet would be achieved in the longer term. Evidence from the literature (Harb *et al.*, 1985; Chamberlain and Choung, 1995; Forbes and Shariatmadari, 1996; Tolkamp and Kyriazakis, 1997; Allcroft, *et al.*,

1999; Ludden *et al.*, 2002; Forbes, 2002) suggests that animals may attempt to achieve their required diet over a longer time frame (see Chapter 4 for discussion of the literature). This may reflect the evolutionary environment within which ruminants have developed. Indeed, ruminants have evolved within heterogeneous environments where food availability is not completely predictable. The ability to tolerate fluctuations in nutrient supply over a few days or longer and to make choices about how much to eat of each of the many foods available may have proved advantageous to animals living in such environments. This evolutionary pressure would likely have led to the development of intake and diet choice regulation mechanisms that best enabled animals to cope with the vagaries of the natural world. Therefore, a regulation mechanism which operates in the medium-term, as a proxy for the long-term would seem to be in agreement with the evidence presented within this thesis and also be adaptive to the natural world within which cows have evolved.

6.4. Implications of the evidence from analysis of short-term feeding behaviour

The efficiency of animal production systems is greatly influenced by the quantity of food that animals eat (Mertens, 1994). Therefore, in order to predict animal performance it is necessary to have robust models that are able to predict feed intake under a variety of conditions (Mertens, 1996; Yearsley *et al.*, 2001). For effective feed intake prediction models to be developed it is necessary to have a robust theory of intake regulation from which predictions of intake can be made (Forbes *et al.*,

1986). Over the last few decades many theories of intake regulation have been postulated (e.g., Conrad, 1966; Forbes, 1980; Tolkamp and Ketelaars, 1992; Mertens, 1994; Mertens, 1996; Emmans, 1997). These theories encompass many different ideas about how intake is regulated, some of which predict that intake is regulated in the short-term. The analysis presented within this thesis therefore provides new information with which to explore current theories of intake regulation.

6.4.1. Theories of diet regulation

Theories of intake regulation can broadly be grouped into one of two types. One group of theories is based on the idea that animals will attempt to eat a diet that allows them to meet their genetic potential, e.g. for growth or milk yield, subject to constraints. An alternative group of theories suggest that animals will optimise, rather than maximise, their intake in order to minimise any costs, relative to benefits, associated with food intake. These two approaches will be examined in greater detail below and the results from analysis of short-term feeding behaviour will be compared and contrasted with the predictions from these theories.

6.4.1.1. Maximisation of intake

The idea that animals maximise their intake subject to constraints so that they can perform at their genetic potential has been implicitly assumed or actually stated in

most work on the subject of intake regulation to date (Yearsley *et al.*, 2001; Ketelaars and Tolkamp, 1992a) and goes back at least to Adolph (1947) (as cited by Emmans and Kyriazakis, 2001). This is based on the idea that animals have a genetically determined potential for production, i.e. growth, reproduction, lactation etc, which they will attempt to achieve (Mertens, 1994). If animals are observed to produce less well than expected then this will be attributed to some type of constraint, e.g. one which limits the animal from eating sufficiently to perform maximally (Conrad, 1966; Mertens, 1994; Poppi *et al.*, 1994; Emmans and Kyriazakis, 1995; Weston, 1996).

Within the context of ruminants, intake was initially thought to be affected solely by the physical bulk of the foods with Blaxter *et al.* (1961) finding that sheep ate to a constant distension of their digestive tract measured by fill. Evidence for this idea was presented in the form of experiments during which the digestive capacity was manipulated by addition of indigestible bulk, often in the form of plastic cubes (Carr and Jocobson, 1966; Baumont *et al.*, 1990) or water filled balloons (Johnson and Combs, 1991; Johnson and Combs, 1992). The evidence from such studies was not always consistent, with Baumont *et al.* (1990) considering the evidence in favour of short-term physical bulk constraints and other authors finding no evidence for this mechanism (Carr and Jocobson, 1966; Johnson and Combs, 1992). Later work has suggested that insertion of balloons into the digestive tract can influence not only the volume available for processing food but also other aspects of digestive function that may affect intake (see Grovum, 1986, Pittroff and Kothmann, 1999; and Tolkamp, 1999 for reviews).

The idea of physical factors regulating intake was subsequently reassessed and thought to apply only to the intake of poorer quality foods. Metabolic factors were proposed to limit intake of more digestible foods in order to prevent ruminants that were performing at their metabolic maximum from over consuming food (Conrad *et al.*, 1964). This work centred on identifying the critical point at which physical constraints disappeared and metabolic regulation appeared. Conrad *et al.* (1964) concluded that with foods above 66.7% digestibility intake was controlled metabolically and below this intake was constrained by dietary bulk. This work led to the idea that physical and metabolic mechanisms work separately in regulating intake depending on the quality of the diet (Pittroff and Kothmann, 1999). The basis of Conrad *et al.* (1964) conclusions have, however, been strongly criticised in light of findings that the statistical analysis used in this study was seriously flawed (Ketelaars and Tolkamp, 1992a; Weston, 1996; Tolkamp, 1999).

Later work eroded the idea of a critical point at which physical constraints stops and metabolic constraints begin (Forbes, 1980; Pittroff and Kothmann, 1999). Therefore, models were further developed in an attempt to represent the mechanisms used by animals to collate the information from the many interrelating intake control factors. As more factors affecting intake have been identified these models have included more and more factors linked to diet regulation. These include the effects of capacity to use energy, digestive capacity, environmental temperature, foraging time constraint, jaw muscle fatigue, abdominal fat limiting intake, faecal output, food palatability, milk production and many others (e.g., Forbes, 1977b; Kristensen, 1986;

Fisher *et al.*, 1987; Poppi *et al.*, 1994; Forbes, 1995a; Weston, 1996; Illius and Jessop, 1996; Iason *et al.*, 1999; Kohlmann *et al.*, 1999; Ingvartsen and Andersen, 2000).

Mechanistic models have, therefore, provided many conflicting reports as to the factors that are key to understanding intake regulation. These approaches may provide some insight into aspects that affect intake, however, Yearsley *et al.* (2001) suggested that an attempt to model every causal link involved in food intake behaviour is futile. Additionally, as intake prediction models have become more complex so the original, and falsifiable, theory of intake maximisation subject to constraints has been diluted in an attempt to model many interacting feed intake predictors (Emmans and Kyriazakis, 2001). Therefore, complicated mechanistic models often suffer a major and fundamental flaw if proposed as theories of intake regulation, i.e. theories resulting from such complex mechanistic models are largely not falsifiable. This is a consequence of the fact that any failure to accurately predict intake by a model can always be, and often has been (Mertens and Ely, 1979; Fisher *et al.*, 1987; Fisher, 1996; Pittroff and Kothmann, 2001a,b,c), attributed to a failure to include one more constraints or interrelated factors into the model.

Within the ecology literature a falsifiable model of intake regulation has also been presented. Optimal foraging theory predicts that animals will maximise their fitness by maximising their energy intake (Stephens and Krebs, 1986). This theory, although developed independently, is essentially similar to the intake maximisation model presented within animal science. In both models animals are assumed to maximise

intake (or some aspect of intake, e.g. energy) subject to constraints. Many of the constraints within optimal foraging theory are also similar to those proposed within animal science. These include; physical and structural properties of the vegetation, i.e. bulk density, surface height, and vertical distribution of biomass (see Illius *et al.*, 1999), daily foraging time, bite rate and digestive constraints (Iason *et al.*, 1999; Shipley *et al.*, 1999). Similarly to animal science, optimal foraging theory has been investigated by the construction of mechanistic models (Belovsky, 1978; Armstrong *et al.*, 1997a,b; Farnsworth and Illius, 1998; Illius and Gordon, 1991; Shipley *et al.*, 1999), however these suffer from similar limits to their applicability as the mechanistic models presented within the animal science literature. This is again a consequence of the complexity of the many interrelating factors that influence intake and our incomplete knowledge of them. Therefore, such models are limited in their usefulness due to incomplete knowledge with which to parameterise them.

Models based on intake maximisation subject to constraints, in their many different forms, have a number of common features. The basis of all these models is the theory that intake is limited in the short-term by mechanisms which constrain intake. If animals are fed good quality diets then they are assumed to consume a diet that provides sufficient nutrients for them to perform at their genetically defined maximum. Intake of good quality foods, which is not limited by physical constraints, are frequently thought to be controlled by short-term depletion-repletion mechanisms (Le Magnen, 1985). Therefore, the nutrients consumed during a meal are used by the animals during the non-feeding between meal interval. The length of this interval is a result of short-term feedback systems that indicate when the animal has used the

nutrients from the previous meal and is therefore deficient. This results in the termination of the non-feeding interval with the beginning of the next meal. Such a feeding strategy is expected to result in a strong correlation between the length of the non-feeding interval and the size of the meal (and/or *vice versa*). Additionally, when offered a choice of foods animals may be expected to select foods which result in a consistent short-term diet such that the nutrients supplied to the animal are those which enable the animal to perform maximally. In contrast, if animals consume foods that are of poorer quality, and thus do not perform at a level that is possible when fed better quality foods, then this is assigned to animals being physically constrained from consuming sufficient foods. Therefore, if intake is constrained in the short-term yet animals are expected to be eating to meet requirements then they would be expected to adjust their daily eating pattern to maintain a constant (maximal) level of rumen fill (Dulphy and Demarquilly, 1994; Forbes, 1995a; Tolkamp *et al.*, 2002). Therefore, if intake maximisation subject to constraints models capture the regulation mechanisms of bulky foods then one would expect to find evidence that short-term feeding behaviour is organised in such a way that constraints are minimised and intake maximised. Additionally, if offered a choice of foods animals may be expected to select that combination of food which maximises the nutritional quality of the diet consumed. Therefore, if animals attempt to consume a diet that supplies nutrients that enable them to perform maximally then this should be evident from analysis of their short-term feeding behaviour.

In contrast, the short-term feeding behaviour results presented above are not in agreement with the expectations from the intake maximisation subject to constraints

theory. Indeed, there is little evidence to support the idea that intake is regulated in the short-term. This conclusion is further supported by the finding of Tolkamp *et al.* (2002) where it was shown that cows fed a high forage, as opposed to those fed a high concentrate, diet had poorer performance, yet despite this they did not differ in their feeding patterns. This indicates that these animals did not attempt to overcome a physical bulk constraint (which was assumed to cause the lower level of performance) by eating regular meals throughout the day and night, thereby ensuring maximal intake. A similar test of these short-term feeding behaviour predictions from the constraint theory was conducted by Whittemore *et al.* (2002) with pigs. They fed pigs either a low or a higher bulk diet and investigated if pigs attempted to overcome the bulk constraint by altering their diurnal feeding patterns. The conclusion from this work was that although pigs ate bulky foods slower than the control food and ate for longer each day they did not extend their eating into the night. Therefore, it appears that these animals did not attempt to overcome short-term constraints by altering their diurnal feeding behaviour in an attempt to maximise intake. This agrees with other observations where animals are clearly not maximising their intake. For example, when animals are fed poor quality diets they are assumed to be maximising intake subject to a physical fill constraint. However, if the atmospheric temperature declines these animals are generally observed to increase intake substantially. It is very hard to argue that the supposed physical limit to intake at the initial temperature was in fact constraining intake (see Illius *et al.* (2002) for a recent review of this issue). Observations such as these raise serious questions about whether animals are maximising intake subject to constraints. As the evidence presented above shows there is little evidence of constraints operating in

the short-term except in more extreme situations, e.g. with high cow pressure per feeder. In summary, little evidence from the analysis of short-term feeding behaviour supports the theory that intake is regulated in the short-term by physical and metabolic constraints.

The conclusion that short-term feeding behaviour is unlikely to often be deterministically controlled must also lead us to further examine mechanistic models that attempt to model short-term feeding behaviour in order to predict food intake. This approach was advocated by Booth (1978), and subsequently by Forbes (1980), to describe the short-term flow of energy from consumed foods, and use this to predict the short-term feeding behaviour of animals. However, if the short-term feeding behaviour of animals cannot be described as deterministic and therefore does not appear to be controlled by short-term regulation processes then the appropriateness of models, which attempt to predict intake based on this premise must be seriously questioned. If the mechanisms used by animals to regulate food intake aim to achieve a consistent diet in the medium-term then any modelling of short-term feeding behaviour is unlikely to reflect the observed behaviour of animals especially if short-term feeding behaviour is very flexible. This approach to modelling intake must therefore be treated with caution.

6.4.1.2. Optimisation of intake

Optimisation of intake in order to maximise efficiency (i.e. the benefit to cost ratio of food intake) has been presented as an alternative, and faslifiable, theory of intake

regulation (Ketelaars and Tolkamp, 1992a,b; Tolkamp and Ketelaars, 1992). In their series of papers Tolkamp and Ketelaars argued it was illogical that animals, which have evolved in an environment where their sole nutrition has been derived from grazing fibrous herbage, would often be constrained by the physical bulk of the forage. They suggested that evolutionary pressure would have ensured that such short-term constraints rarely influenced intake. This dissatisfaction with constraints theories led to the proposal of an alternative theory of intake regulation which encompassed the idea that foods not only have a positive benefit, i.e. provide nutrients, but also have a cost associated with them. This cost was thought to be a consequence of oxygen intake, which is a necessary part of processing foods into energy, but results in the production of oxygen free radicals. These are known to cause intracellular damage and are linked with a decrease in fitness (see Tolkamp and Ketelaars, 1992 for discussion). Food intake regulation could then aim to maximise the benefit to cost ratio of consuming food, i.e. net energy intake per unit of oxygen consumed such that evolutionary fitness was maximised (Tolkamp and Ketelaars, 1992). Therefore, in contrast to previous theories, animals may be expected to consume a sub-maximal amount of food if this improves long-term fitness.

Critics of the optimisation approach taken by Tolkamp and Ketelaars however argue that this approach is not consistent with the evidence. Indeed, Fisher (2002) suggested that animals could simply reduce free radical damage by consuming less food, and as a consequence longevity would be increased. This however, implies that longevity and evolutionary fitness are synonymous. The evidence for this is less

apparent. The theory of Tolkamp and Ketelaars has also been criticised by Mertens (1996), where the theory is interpreted as a feedback control mechanism, which is analogous to fill, rather than as an underlying mechanism on which intake is regulated. The most rigorous challenge to the optimisation approach taken by Tolkamp and Ketelaars has been presented by Emmans and Kyriazakis (1995). In brief, Emmans and Kyriazakis (1995) argue that Tolkamp and Ketelaars optimisation theory is incompatible with expectations for growing animals. Indeed, they suggest a direct consequence of animals maximising the net energy intake per unit of oxygen consumed is that animals can be predicted to continue to grow indefinitely, rather than maintaining a mature body weight. Therefore, this approach remains contentious.

More recently Forbes, (2001) presented a model that described how animals may attempt to minimise their 'discomfort' by optimising the intake of various components of their diet within the boundaries set by their genetically determined potential for production. Here the cost to benefit ratio was between the costs of discomfort, e.g. due to excess consumption of fibre, lack of metabolizable energy or cost of time spent grazing, as opposed the benefits of food intake. In this model optimum intake may also be less than the maximum possible. Although this theory is not properly quantified (Forbes, 2002) it demonstrates that, at least in theory, an optimisation model similar to that proposed by Tolkamp and Ketelaars (1992) may provide an alternative approach to the intake maximisation theories.

Illius *et al.* (2002) have recently explored the control of feed intake and assessed the merits of requirements and optimisation theories from an evolutionary perspective. They propose that animals attempt to optimise the fitness function of food intake. This fitness function comprises the positive benefits of food which increase evolutionary fitness, e.g. survival, growth and reproduction, and the costs of intake, e.g. exposure to predators, parasites and food toxins. Although the currency of short or medium-term optimisation is not made explicit within this study, and is therefore not falsefiable, optimal intake is proposed as a balance of the benefits and costs of food intake with the aim of maximising long-term fitness.

These optimisation theories have provided a serious challenge to the long-standing constraints theory. Therefore, considerable debate has been promoted within the field of intake regulation, resulting in both strong criticisms of optimisation theories and acknowledgement that a new approach may be beneficial due to apparent weaknesses of current theories (Mertens, 1994; Emmans and Kyriazakis, 1995; Forbes, 1995a; Ketelaars and Tolkamp, 1996; Tolkamp, 1999; Fisher, 2002; Whittemore *et al.*, 2002). In light of the approach taken within this thesis, a weakness of these optimisation models is the lack of explicit predictions about short-term feeding behaviour. It is however generally assumed that flexibility in the short-term will be expected if animals optimise their diet in the medium-term as a proxy for long-term fitness maximisation. Despite this it is not possible, in contrast to constraints theories, to use short-term feeding behaviour to falsify optimisation theories.

6.5. General conclusions

For animals to have survived within heterogeneous environments the ability to regulate both the composition and the quantity of food consumed must have been of immense value. It therefore seems highly likely that animals would have evolved mechanisms with which to regulate their diet. The obvious question then is, what are the mechanisms used to regulate intake and diet composition? Evidence from the literature and from the work presented in this thesis suggests that animals do not use very short-term mechanisms to achieve a diet that is the same in the short-term as that consumed across long periods. Short-term deterministic regulation does not therefore seem to be the predominant method used by animals to regulate their diet. This finding has considerable implications for proposed theories of intake regulation, which have often made implicit assumptions that intake is regulated within the short-term. Indeed, this work suggests that although intake must be constrained by aspects of the food, i.e. bulk, under some conditions, the importance of such constraints have likely been over estimated in the literature. Further to this, the development of mechanistic models which attempt to capture the many interrelating factors that are thought to influence intake regulation must be reassessed. These models are currently of limited value due to our incomplete knowledge of the mechanisms regulating intake and hence our inability to accurately parameterise them.

Recently, increasing evidence, which this theses contributes to, is leading to a reassessment of the mechanisms which have long been thought to control intake. As a consequence, authors are increasingly searching for new ideas about how intake is

regulated. Such work is focussing more heavily on regulation mechanisms that operate with a medium-term goal as a proxy for long-term intake regulation mechanisms. This approach would seem intuitively to be more appropriate to animals that have evolved in unpredictable environments where flexibility is very important. The role of optimisation mechanisms are therefore being increasingly considered as important within food intake regulation. However, as yet there is no consensus on the currency with which animals optimise their intake and diet. Indeed, this could be based on time, although whether this would be clock time, metabolic time or some other measure of time is unclear. Further, animals may attempt to regulate intake and diet composition such that they stay within physiologically determined limits. Therefore, animals may select a diet which, although vary variable in the short-term, may enable a consistency to be maintained in the medium-term. Optimisation theories now need to be further developed to account for the observed feeding behaviour of animals before prediction of intake, based on sound biological principles, can be developed.

6.6. Future work

Historically, the study of food intake regulation within animal and ecological sciences has often proceeded along very different routes. Within animal science a reductionist approach to understanding the mechanisms which affect intake has generally been favoured. Therefore, animal scientists have devoted considerable effort to the question of how intake and diet composition is regulated. In contrast to

this, within the ecology literature a longer-term perspective has generally been adopted. This has necessarily led to questions about why intake is regulated and how this relates to the evolutionary fitness of animals. These two separate branches of science do however have at least one common desire, that is for a better understanding of the mechanisms which animals use to regulate intake. Such knowledge would have a profound impact on our management and understanding of animal systems and how these interact with the natural world. Future work may therefore be strengthened by more effective communication between these two disparate branches of science. Indeed, bringing a lifetime fitness perspective to the detailed reductionist thinking which is typical of animal scientists may enable a much-needed breakthrough to be made.

As part of this new approach to analysing feeding behaviour the conclusion of this thesis, that evolution has likely favoured the adoption of medium-term proxies for intake regulation, need to be further developed. The most pressing questions to be tackled are, what is/are the medium-term proxies which animals use to regulate their diet and how can they be identified? To this end, the approach to studying short-term feeding behaviour used within this thesis, i.e. detailed mathematical analysis, should be central to future work which aims to identify patterns in feeding behaviour. This may therefore provide valuable evidence with which to develop new ideas about intake and diet composition regulation.

BIBLIOGRAPHY

Adenuga, M. K., B. J. Tolkamp, A. A. Ademosun, G. Montsma, and B. O. Brouwer. 1991. Effect of pregnancy and lactation on liveweight, feed intake and feeding behavior in West African Dwarf (WAD) goats. Small Ruminant Research 4:245-255.

AFRC. 1993. Energy and protein requirements of ruminants. Advisory manual prepared by AFRC technical committee on responses to nutrients. CAB International, Wallingford.

Allcroft, D. J., C. A. Glasbey, I. Kyriazakis, and B. J. Tolkamp. 1999. What is the critical time scale of a cow's feeding behaviour? Proceedings of the Thirty-first Meeting of the Agricultural Research Modellers' Group. J France and LA Crompton (Eds). Journal of Agricultural Science 133: 344-345.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83:1598-1624.

Al-Rabbat, M. F., R. L. Baldwin, and W. C. Weir. 1970. Microbial growth dependence on ammonia nitrogen in the bovine rumen: A quantitative study. Journal of Dairy Science 54:1162-1172.

Armitage, P. and T. Colton (Eds.). 1998. Encyclopaedia of Biostatistics. Chichester: Wiley.

Armstrong, H. M., I. J. Gordon, S. A. Grant, N. J. Hutchings, J. A. Milne, and A. R. Sibbald. 1997a. A model of the grazing of hill vegetation by sheep in the UK .1. The prediction of vegetation biomass. Journal of Applied Ecology 34:166-185.

Armstrong, H. M., I. J. Gordon, N. J. Hutchings, A. W. Illius, J. A. Milne, and A. R. Sibbald. 1997b. A model of the grazing of hill vegetation by sheep in the UK .2. The prediction of offtake by sheep. Journal of Applied Ecology 34:186-207.

Arsenos, G., G. C. Emmans, and W. Kyriazakis. 2000. Variation between individuals and the consequences for diet selection by groups of animals. Animal Behaviour 60:811-820.

Atwood, S. B., F. D. Provenza, R. D. Wiedmeier, and R. E. Banner. 2001. Influence of free-choice vs mixed-ration diets on food intake and performance of fattening calves. Journal of Animal Science 79:3034-3040.

Barrio, J. P., S. Y. Zhang, Z. K. Zhu, F. L. Wu, X. Z. Mao, F. F. Bermudez, and J. M. Forbes. 2000. The feeding behaviour of the water buffalo monitored by a semiautomatic feed intake recording system. Journal of Animal and Feed Sciences 9:55-72.

Baumont, R., C. H. Malbert, and Y. Ruckebusch. 1990. Mechanical stimulation of rumen fill and alimentary behavior in sheep. Animal Production 50:123-128.

Beauchemin, K. A., M. Maekawa, and D. A. Christensen. 2002. Effect of diet and parity on meal patterns of lactating dairy cows. Canadian Journal of Animal Science 82:215-223.

Belovsky, G. E. 1978. Diet optimization in a generalist herbivore: The moose. Theoretical Population Biology 14:105-134.

Berdoy, M. 1993. Defining bouts of behaviour - a 3-process model. Animal Behaviour 46:387-396.

Bermudez, F. F., J. M. Forbes, and R. Jones. 1989. Feed intakes and meal patterns of sheep during pregnancy and lactation, and after weaning. Appetite 13:211-222.

Berthoud, H. R. 2002. Multiple neural systems controlling food intake and body weight. Neuroscience and Biobehavioural Reviews 26:393-428

Bigelow, J. A. and T. R. Houpt. 1988. Feeding and drinking patterns in young-pigs. Physiology and Behavior 43:99-109.

Blaxter, K. L., F. W. Wainman, and R. S. Wilson. 1961. The regulation of food intake by sheep. Animal Production 3:51-61.

Booth, D.A. Prediction of feeding behaviour from energy flows in the rat. 1978. In: Hunger models: Computable theory of feeding control. D. A. Booth (ed). London: Academic Press.

Bornett, H. L. I., C. A. Morgan, A. B. Lawrence, and J. Mann. 2000. The flexibility of feeding patterns in individually housed pigs. Animal Science 70:457-469.

Burden, R. L. and Faires, J. D. 1985. Numerical Analysis. pp. 28. Prindle, Weber and Schmidt, Boston.

Carr, S. B. and D. R. Jocobson. 1966. Intraruminal addition of mass or removal of rumen contents on voluntary intake of the bovine. Journal of Dairy Science 50:1814-1818.

Chamberlain, D. G. and J. J. Choung. 1995. The importance of rate of ruminal fermentation of energy sources in diets for dairy cows. In: Recent Advances in Animal Nutrition. P. C. Garnsworthy and D. J. A. Cole (Eds.). pp. 3-27. Nottingham University Press, Nottingham.

Chambers, P. G., S. J. Simpson, and D. Raubenheimer. 1995. Behavioural mechanisms of nutrient balancing in Locusta migratoria nymphs. Animal Behaviour 50:1513-1523.

Church, D. C. 1969. Digestive physiology and nutrition of ruminants. D.C. Church, Oregon.

Cox, D.R. and D. Oakes. 1984. Analysis of Survival Data. London: Chapman & Hall.

Collier, G. and D. F. Johnson. 1990. The time window of feeding. Physiology and Behavior 48:771-777.

Collier, G., D. F. Johnson, and C. Mitchell. 1999. The relation between meal size and the time between meals: Effects of cage complexity and food cost. Physiology and Behavior 67:339-346.

Conrad, H. R. 1966. Symposium on factors influencing the voluntary intake of herbage by ruminants: Physiological and physical factors limiting feed intake. Journal of Animal Science 25:227-235.

Conrad, H. R., A. D. Pratt, and J. W. Hibbs. 1964. Regulation of feed intake in dairy cows. 1. Changes in importance of physical and physiological factors with increasing digestibility. Journal of Dairy Science 47:54-62.

Dado, R. G. and M. S. Allen. 1994. Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy-cows. Journal of Dairy Science 77:132-144.

Davis, J. D. 1996. Deterministic and probabilistic control of the behavior of rats ingesting liquid diets. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 39:R793-R800.

Day, J. E. L., I. Kyriazakis, and P. J. Rogers. 1998. Food choice and intake: towards a unifying framework of learning and feeding motivation. Nutrition Research Reviews 11:25-43.

de Castro, J. M. 1975. Meal pattern correlations: Facts and artefacts. Physiology & Behavior 15:13-15.

de Haer, L. C. M. and J. W. M. Merks. 1992. Patterns of daily food-intake in growing pigs. Animal Production 54:95-104.

Demaria-Pesce, V. H. and S. Nicolaidis. 1998. Mathematical determination of feeding patterns and its consequence on correlational studies. Physiology and Behavior 65:157-170.

Dewsbury, D. A. 1983. Recovery from sexual satiety in Deer Mice (*peromyscus-maniculatus bairdi*). Journal of Comparative Psychology 97:34-42.

Dulphy, J. P. and C. Demarquilly. 1994. The regulation and prediction of feed-intake in ruminants in relation to feed characteristics. Livestock Production Science 39:1-12.

Dürr, J. W., H. G. Monardes, and R. I. Cue. 1999. Genetic analysis of herd life in Quebec Holsteins using Weibull models. Journal of Dairy Science 82:2503-2513.

Dürst, B., M. Senn, and W. Langhans. 1993. Eating patterns of lactating dairy-cows of 3 different breeds fed grass *ad-lib*. Physiology and Behavior 54:625-631.

Elizalde, H. F. 1993. Studies on the effects of chemical and physical characteristics of grass silage and degree of competition per feeding space on the feeding behaviour of lactation dairy cows. PhD thesis, Univ. of Belfast.

Elizalde, H. F. and C. S. Mayne. 1993. The effect of degree of competition per feeding space on the dry matter intake and eating behaviour of dairy cows offered grass silage. Pages 137-138 in Proc. 3rd Research Conf., Brit. Grassland Soc., Greenmont College.

Ellis, T.M.R., Philips, I.R. & Lahey, T.M. (1994). *Fortran 90 programming*. Harlow: Addison-Wesley.

Ellis, W. C., D. Poppi, and J. H. Matis. 2000. Feed intake in ruminants: Kinetic aspects. In: Farm animal metabolism and nutrition. J. P. F. D'Mello (Ed.). pp. 335-363. CAB International, Wallingford.

Emmans, G. C. 1997. A method to predict the food intake of domestic animals from birth to maturity as a function of time. Journal of Theoretical Biology 186:189-199.

Emmans, G. C. and I. Kyriazakis. 1995. The idea of optimisation in animals: Uses and dangers. Livestock Production Science 44:189-197.

Emmans, G. C. and I. Kyriazakis. 2001. Consequences of genetic change in farm animals on food intake and feeding behaviour. Proceedings of the Nutrition Society 60:115-125.

Everitt, B.S. 1998. Dictionary of Statistics. Guildford: Cambridge University Press.

Farnsworth, K. D. and A. W. Illius. 1998. Optimal diet choice for large herbivores: an extended contingency model. Functional Ecology 12:74-81.

Fisher, D. S. 1996. Modeling ruminant feed intake with protein, chemostatic, and distention feedbacks. Journal of Animal Science 74:3076-3081.

Fisher, D. S. 2002. A review of a few key factors regulating voluntary feed intake in ruminants. Crop Science 42:1651-1655.

Fisher, D. S., J. C. Burns, and K. R. Pond. 1987. Modeling ad-libitum dry-matter intake by ruminants as regulated by distension and chemostatic feedbacks. Journal of Theoretical Biology 126:407-418.

Forbes, J. M. 1977a. Development of a model of voluntary food intake and balance in lactation cows. Animal Production 24:203-214.

Forbes, J. M. 1977b. Interrelationships between physical and metabolic control of voluntary food intake in fattening, pregnant and lactating mature sheep: A model. Animal Production 24:91-101.

Forbes, J. M. 1980. A model of the short-term control of feeding in the ruminant - effects of changing animal or feed characteristics. Appetite 1:21-41.

Forbes, J. M. 1983. Models for the prediction of food-intake and energy-balance in dairy-cows. Livestock Production Science 10:149-157.

Forbes, J. M. 1985. The importance of meals in the regulation of food intake. Proceedings of the Nutrition Society of Australia 10:14-24.

Forbes, J. M. 1988. Metabolic aspects of the regulation of voluntary food intake and appetite. Nutrition Research Reviews 1:145-168.

Forbes, J.M. 1995a. Voluntary food intake and diet selection in farm animals. CAB International, Wallingford.

Forbes, J. M. 1995b. Voluntary intake: A limiting factor to production in high-yielding dairy cows? In: Breeding and feeding the high genetic merit dairy cow. Lawrence, T. J. L., F. J., Gordon, and A. Carson (Eds.). British Society of Animal Science S19:13-19

Forbes, J. M. 1999. Minimal total discomfort as a concept for the control of food intake and selection. Appetite 33:371.

Forbes, J. M. 2000. Physiological and metabolic aspects of feed intake control. In: Farm animal metabolism and nutrition. J. P. F. D'Mello (Ed.). pp. 319-333. CAB International, Wallingford.

Forbes, J. M. 2001. Consequences of feeding for future feeding. Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology 128:463-470.

Forbes, J. M. 2002. The multifactorial nature of food intake control. Journal of Animal Science 80 (S1):112.

Forbes, J. M., D. A. Jackson, C. L. Johnson, P. Stockill, and B. S. Hoyle. 1986. A method for the automatic monitoring of food-intake and feeding-behavior of individual cattle kept in a group. Research and Development in Agriculture 3:175-180.

Forbes, J. M. and I. Kyriazakis. 1995. Food preferences in farm animals: why don't they always choose wisely? Proceedings of the Nutrition Society 54:429-440.

Forbes, J. M. and F. Shariatmadari. 1994. Diet selection for protein by poultry. Worlds Poultry Science Journal 50:7-24.

Forbes, J. M. and F. Shariatmadari. 1996. Short-term effects of food protein content on subsequent diet selection by chickens and the consequences of alternate feeding of high- and low-protein foods. British Poultry Science 37:597-607.

Friggens, N. C., B. L. Nielsen, I. Kyriazakis, B. J. Tolkamp, and G. C. Emmans. 1998. Effects of feed composition and stage of lactation on the short-term feeding behavior of dairy cows. Journal of Dairy Science 81:3268-3277.

Frische, S., A. Fago, and J. Altimiras. 2000. Respiratory responses to short term hypoxia in the snapping turtle, Chelydra serpentina. Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology 126:223-231.

Galef, B. G. 1991. A contrarian view of the wisdom of the body as it relates to dietary self-selection. Psychological Review 98:218-223.

Gill, M. and D. L. Romney. 1994. The relationship between the control of meal size and the control of daily intake in ruminants. Livestock Production Science 39:13-18.

GonzalezMariscal, G., M. E. Albonetti, E. Cuamatzi, and C. Beyer. 1997. Transitory inhibition of scent marking by copulation in male and female rabbits. Animal Behaviour 53:323-333.

Grovum, W. L. 1986. A new look at what is controlling food intake. In: Feed intake by Beef Cattle. F. N. Owen (Ed.). pp 1-39. Oklahoma State University.

Gupta, R. D. and D. Kundu. 1999. Generalized exponential distributions. Australian and New Zealand Journal of Statistics 41:173-188.

Harb, M. Y., V. S. Reynolds, and R. C. Campling. 1985. Eating behavior, social-dominance and voluntary intake of silage in group-fed milking cattle. Grass and Forage Science 40:113-118.

He, X. M. and W. K. Fung. 1999. Method of medians for lifetime data with Weibull models. Statistics in Medicine 18:1993-2009.

Iason, G. R., A. R. Mantecon, D. A. Sim, J. Gonzalez, E. Foreman, F. F. Bermudez, and D. A. Elston. 1999. Can grazing sheep compensate for a daily foraging time constraint? Journal of Animal Ecology 68:87-93.

Illius, A. W. and I. J. Gordon. 1991. Prediction of intake and digestion in ruminants by a model of rumen kinetics integrating animal size and plant characteristics. Journal of Agricultural Science 116:145-157.

Illius, A. W., I. J. Gordon, D. A. Elston, and J. D. Milne. 1999. Diet selection in goats: A test of intake-rate maximization. Ecology 80:1008-1018.

Illius, A. W. and N. S. Jessop. 1996. Metabolic constraints on voluntary intake in ruminants. Journal of Animal Science 74:3052-3062.

Illius, A.W., Tolkamp, B.J. and Yearsley, J. 2002. The evolution of the control of food intake. Proceedings of the Nutrition Society. 61: 465-472.

Ingvartsen, K. L. and J. B. Andersen. 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. Journal of Dairy Science 83:1573-1597.

Johnson, N. L., S. Kotz, and N. Balakrishnan. 1994. Continuous univariate distributions. John Wiley and Sons, New York.

Johnson, T. R. and D. K. Combs. 1991. Effects of prepartum diet, inert rumen bulk, and dietary polyethylene-glycol on dry-matter intake of lactating dairy-cows. Journal of Dairy Science 74:933-944.

Johnson, T. R. and D. K. Combs. 1992. Effects of inert rumen bulk on dry-matter intake in early and midlactation cows fed diets differing in forage content. Journal of Dairy Science 75:508-519.

Kachman, S. D. 1999. Applications in survival analysis. Journal of Animal Science 77:147-153.

Ketelaars, J. J. M. H. and B. J. Tolkamp. 1992a. Toward a new theory of feed intake regulation in ruminants. 1. Causes of differences in voluntary feed intake: critique of current views. Livestock Production Science 30:269-296.

Ketelaars, J. J. M. H. and B. J. Tolkamp. 1992b. Toward a new theory of feed intake regulation in ruminants. 3. Optimum feed intake: in search of a physiological background. Livestock Production Science 31:235-258.

Ketelaars, J. J. M. H. and B. J. Tolkamp. 1996. Oxygen efficiency and the control of energy flow in animals and humans. Journal of Animal Science 74:3036-3051.

Kim, K. H., J. J. Choung, and D. G. Chamberlain. 1999a. Effects of varying the degree of synchrony of energy and nitrogen release in the rumen on the synthesis of microbial protein in lactating dairy cows consuming a diet of grass silage and a cereal-based concentrate. Journal of the Science of Food and Agriculture 79:1441-1447.

Kim, K. H., Y. G. Oh, J. J. Choung, and D. G. Chamberlain. 1999b. Effects of varying degrees of synchrony of energy and nitrogen release in the rumen on the synthesis of microbial protein in cattle consuming grass silage. Journal of the Science of Food and Agriculture 79:833-838.

Kleinbaum, D. G., L. L. Kupper, and K.E. Muller. 1988. Applied Regression Analysis and Other Multivariate Methods. Boston: PWS-Kent.

Kohlmann, S. G., J. H. Matis, and K. L. Risenhoover. 1999. Estimating handling times for herbivore diets: a statistical method using the gamma distribution. Journal of Animal Ecology 68:638-645.

Kristensen, A. R. 1986. A model to describe and predict the production and food intake of the dairy cow. Acta Agriculturae Scandinavica 36:305-317.

Kyriazakis, I. 1997. The nutritional choices of farm animals: to eat or what to eat? In Animal Choices. Forbes, J. M., T. J. L. Lawrence, R. G. Rodway. and M. A. Varley (Eds.). pp 55-65. British Society of Animal Science S20:55-65

Kyriazakis, I. and J. E. L. Day. 1998. Does the study of feeding behaviour benefit from a teleonomic framework? Nutrition Research Reviews 11:223-229.

Kyriazakis, I. and G. C. Emmans. 1990. The immediate effects of abrupt diet composition changes in young-pigs. British Journal of Nutrition 64:619-623.

Kyriazakis, I. and G. C. Emmans. 1991. Diet selection in pigs - dietary choices made by growing pigs following a period of underfeeding with protein. Animal Production 52:337-346.

Kyriazakis, I. and G. C. Emmans. 1999. Voluntary food intake and diet selection. In: A quantitative biology of the pig. I. Kyriazakis (Ed.). pp. 229-248. CABI publishing, Oxon.

Kyriazakis, I., G. C. Emmans, and C. T. Whittemore. 1990. Diet selection in pigs - choices made by growing pigs given foods of different protein concentrations. Animal Production 51:189-199.

Kyriazakis, I. and J. D. Oldham. 1993. Diet selection in sheep: the ability of growing lambs to select a diet that meets their crude protein (nitrogen X 6.25) requirements. British Journal of Nutrition 69:617-629.

Kyriazakis, I., B. J. Tolkamp, and G. Emmans. 1999. Diet selection and animal state: an integrative framework. Proceedings of the Nutrition Society 58:765-771.

Langton, S. D., D. Collett, and R. M. Sibly. 1995. Splitting behaviour into bouts - a maximum-likelihood approach. Behaviour 132:781-799.

Larue-Achagiotis, C., C. Martin, P. Verger, and J. Louis-Sylvestre. 1992. Dietary self-selection vs. complete diet - body-weight gain and meal patterns in rats. Physiology and Behavior 51:995-999.

Le Magnen, J. 1985. Hunger. Cambridge University Press, Cambridge.

Le Magnen, J. and M. Devos. 1980. Parameters of the meal pattern in rats: their assessment and physiological significance. Neuroscience and Biobehavioral Reviews 4:1-11.

Leger, D. W. and I. A. Didrichsons. 1994. An assessment of data pooling and some alternatives. Animal Behaviour 48:823-832.

Ludden, P. A., T. L. Wechter, and B. W. Hess. 2002. Effects of oscillating dietary protein on nutrient digestibility, nitrogen metabolism, and gastrointestinal organ mass in sheep. Journal of Animal Science 80:3021-3026.

Machlis, L., P. W. D. Dodd, and J. C. Fentress. 1985. The pooling fallacy - problems arising when individuals contribute more than one observation to the data set. Zeitschrift fur Tierpsychologie-Journal of Comparative Ethology 68:201-214.

Martin, P. and H. C. Kraemer. 1987. Individual-differences in behavior and their statistical consequences. Animal Behaviour 35:1366-1375.

Mayes, E. and P. Duncan. 1986. Temporal patterns of feeding-behavior in free-ranging horses. Behaviour 96:105-129.

McDonald, P., R. A. Edwards, J. F. D. Greenhalgh, and C. A. Morgan. 2001. Animal Nutrition. Longman, Harlow.

Mertens, D. R. 1987. Predicting intake and digestibility using mathematical models of ruminal function. Journal of Animal Science 64:1548-1558.

Mertens, D. R. 1994. Regulation of forage intake. In: Forage quality, evaluation and utilization. G. C. Fahey (Ed.). pp. 450-493.

Mertens, D. R. 1996. Methods in modelling feeding behaviour and intake in herbivores. Annales de Zootechnie 45:153-164.

Mertens, D. R. and L. O. Ely. 1979. A dynamic model of fiber digestion and passage in the ruminant for evaluating forage quality. Journal of Animal Science 49:1085-1095.

Metz, J.H.M. 1975. Time Patterns of Feeding and Rumination in Domestic Cattle. Wageningen: Communications Agricultural University.

Minitab. 1998. Minitab for Windows, release 12.1. Minitab, Inc., USA.

Morgan, C. A., G. C. Emmans, B. J. Tolkamp, and I. Kyriazakis. 2000a. Analysis of the feeding behavior of pigs using different models. Physiology and Behavior 68:395-403.

Morgan, C. A., B. J. Tolkamp, G. C. Emmans, and I. Kyriazakis. 2000b. The way in which the data are combined affects the interpretation of short-term feeding behavior. Physiology and Behavior 70:391-396.

Musial, F., A. Kowalski, P. Enck, and K. T. Kalveram. 2000. A computer-controlled long-term recording system for temporal analysis of ingestive and excretory

behaviors in miniature-pigs. International Journal of Psychophysiology 35:54-55.

NAG. 1993. Library Manual Mark 16. Numerical Algorithms Group, NAG central office, 256 Banbury road, Oxford, OX2 7DE, UK.

Newman, J. A., A. J. Parsons, J. H. M. Thornley, P. D. Penning, and J. R. Krebs. 1995. Optimal diet selection by a generalist grazing herbivore. Functional Ecology 9:255-268.

Nia, S. A. M., P. H. Robinson, M. Gill, J. R. Ingalls, and J. J. Kennelly. 1995. Influence of feeding a rapidly rumen degraded dietary protein at night or with a basal mixed ration on performance of dairy cows. Canadian Journal of Animal Science 75:575-582.

Nicholson, J. W. G., E. Charmley, and R. S. Bush. 1992. The effect of supplemental protein-source on ammonia levels in the rumen fluid and blood and intake of alfalfa silage by beef-cattle. Canadian Journal of Animal Science 72:853-862.

Nielsen, B. L. 1999. On the interpretation of feeding behaviour measures and the use of feeding rate as an indicator of social constraint. Applied Animal Behaviour Science 63:79-91.

Nielsen, B. L., A. B. Lawrence, and C. T. Whittemore. 1995. Effects of single-space feeder design on feeding-behavior and performance of growing pigs. Animal Science 61:575-579.

Orr, R. J., P. D. Penning, A. Harvey, and R. A. Champion. 1997. Diurnal patterns of intake rate by sheep grazing monocultures of ryegrass or white clover. Applied Animal Behaviour Science 52:65-77.

Panksepp, J. 1973. Reanalysis of feeding patterns in the rat. Journal of Comparative and Physiological Psychology 82:78-94.

Parker, D. S., M. A. Lomax, C. J. Seal, and J. C. Wilton. 1995. Metabolic implications of ammonia production in the ruminant. Proceedings of the Nutrition Society 54:549-563.

Parsons, A. J., J. A. Newman, P. D. Penning, A. Harvey, and R. J. Orr. 1994. Diet preference of sheep - effects of recent diet, physiological-state and species abundance. Journal of Animal Ecology 63:465-478.

Peng, C.K., Hausdorff, J.M., and Goldberger, A.L. 2000. Fractal mechanisms in neural control: Human heartbeat and gait dynamics in health and disease. In: Self-organized biological dynamics and nonlinear control. J. Walleczek (Ed.) p66-96. Cambridge: Cambridge University Press.

Pittroff, W. and M. M. Kothmann. 1999. Regulation of intake and diet selection by herbivores. In: Nutritional ecology of herbivores. H. J. G. Jung and G. C. Fahey (Eds.). Proceedings of the Vth international symposium on the nutrition of herbivores. pp. 366-422. American Society of Animal Science, Savoy.

Pittroff, W. and M. M. Kothmann. 2001a. Quantitative prediction of feed intake in ruminants I. Conceptual and mathematical analysis of models for sheep. Livestock Production Science 71:131-150.

Pittroff, W. and M. M. Kothmann. 2001b. Quantitative prediction of feed intake in ruminants II. Conceptual and mathematical analysis of models for cattle. Livestock Production Science 71:151-169.

Pittroff, W. and M. M. Kothmann. 2001c. Quantitative prediction of feed intake in ruminants III. Comparative example calculations and discussion. Livestock Production Science 71:171-181.

Pomar, C., Knap, P. W., Kyriazakis, I., and Emmans, G. C. 2002. Modeling stochasticity: dealing with populations rather than individual pigs. Journal of Animal Science 80(S1):174.

Poppi, D., M. Gill, and J. France. 1994. Integration of theories of intake regulation in growing ruminants. Journal of Theoretical Biology 167:129-145.

Poppi, D. P. 1996. Predictions of food intake in ruminants from analyses of food composition. Australian Journal of Agricultural Research 47:489-504.

Provenza, F. D. 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. Journal of Range Management 48:2-17.

Provenza, F. D. 1996. Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. Journal of Animal Science 74:2010-2020.

Provenza, F. D., J. J. Villalba, C. D. Cheney, and S. J. Werner. 1998. Self-organization of foraging behaviour: From simplicity to complexity without goals. Nutrition Research Reviews 11:199-222.

Quiniou, N., S. Dubois, and J. Noblet. 2000a. Voluntary feed intake and feeding behaviour of group-housed growing pigs are affected by ambient temperature and body weight. Livestock Production Science 63:245-253.

Quiniou, N., D. Renaudeau, S. Dubois, and J. Noblet. 2000b. Effect of diurnally fluctuating high ambient temperatures on performance and feeding behaviour of multiparous lactating sows. Animal Science 71:571-575.

Redondo, E., S. Regodon, A. Franco, J. Masot, A. Gazquez, and D. P. Cardinali. 2003. Day-night changes in plasma melatonin levels, synaptophysin expression and ultrastructural properties of pinealocytes in developing female sheep under natural long and short photoperiods. Histology and Histopathology 18:333-342,

Rovee-Collier, C., H. Hayne, G. Collier, P. C. Griesler, and G. B. Rovee. 1996. Diet selection by chicks. Developmental Psychobiology 29:241-272.

Rushing, P. A., T. A. Houpt, R. P. Henderson, and J. Gibbs. 1997. High lick rate is maintained throughout spontaneous liquid meals in freely feeding rats. Physiology and Behavior 62:1185-1188.

Sanderson, J. D. and D. A. Vanderweele. 1975. Analysis of feeding patterns in normal and vagotomized rabbits. Physiology and Behavior 15:357-364.

Savory, C. J. 1981. Correlations between meals and inter-meal intervals in japanese quail and their significance in the control of feeding. Behavioural Processes 6:23-36.

Savory, C. J. 1999. Temporal control of feeding behaviour and its association with gastrointestinal function. Journal of Experimental Zoology 283:339-347.

Schilstra, A. J. 1981. Meal-interval correlations - what can they tell us. Physiology and Behavior 27:299-304.

Schwartzkopf-Genswein, K. S., S. Atwood, and T. A. McAllister. 2002. Relationships between bunk attendance, intake and performance of steers and heifers on varying feeding regimes. Applied Animal Behaviour Science 76:179-188.

Scott, L. L. and F. D. Provenza. 1999. Variation in food selection foods among lambs: Effects of basal diet and offered in a meal. Journal of Animal Science 77:2391-2397.

Shabi, Z., A. Arieli, I. Bruckental, Y. Aharoni, S. Zamwel, A. Bor, and H. Tagari. 1998. Effect of the synchronization of the degradation of dietary crude protein and organic matter and feeding frequency on ruminal fermentation and flow of digesta in the abomasum of dairy cows. Journal of Dairy Science 81:1991-2000.

Shariatmadari, F. and J. M. Forbes. 1992. Feeding behaviour of broiler chickens offered a choice of high- and low-protein foods. pp 470. Proceedings of the 104th British Society of Animal Production, Scarborough.

Shipley, L. A., A. W. Illius, K. Danell, N. T. Hobbs, and D. E. Spalinger. 1999. Predicting bite size selection of mammalian herbivores: a test of a general model of diet optimization. Oikos 84:55-68.

Shorposner, G., G. Brennan, C. Ian, R. Jasaitis, K. Madhu, and S. F. Leibowitz. 1994. Meal patterns of macronutrient intake in rats with particular dietary preferences. American Journal of Physiology 266:R1395-R1402.

Sibly, R. M., H. M. R. Nott, and D. J. Fletcher. 1990. Splitting behavior into bouts. Animal Behaviour 39:63-69.

Simpson, S. J. 1982. Patterns in feeding: a behavioural analysis using Locusta migratoria nymphs. Physiological Entomology 7:325-336.

Simpson, S. J., L. B. Browne, and A. C. M. Vangerwen. 1989. The patterning of compensatory sugar feeding in the Australian sheep Blowfly. Physiological Entomology 14:91-105.

Simpson, S. and A. Ludlow. 1986. Why locusts start to feed: a comparison of causal factors. Animal Behaviour 34:480-496.

Sinclair, L. A., P. C. Garnsworthy, J. R. Newbold, and P. J. Buttery. 1993. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein-synthesis in sheep. Journal of Agricultural Science 120:251-263.

Sinclair, L. A., P. C. Garnsworthy, J. R. Newbold, and P. J. Buttery. 1995. Effects of synchronizing the rate of dietary energy and nitrogen release in diets with a

similar carbohydrate- composition on rumen fermentation and microbial protein- synthesis in sheep. Journal of Agricultural Science 124:463-472.

Slater, P. J. B. 1974. The temporal pattern of feeding in the Zebra finch. Animal Behaviour 22:506-515.

Slater, P. J. B. and N. P. Lester. 1982. Minimizing errors in splitting behavior into bouts. Behaviour 79:153-161.

Stamer, E., E. Junge, and E. Kalm. 1997. Temporal pattern of feeding of dairy cows kept in groups. Archiv fur Tierzucht 40:195-214.

Stephens, D. W. and J. R. Krebs. 1986. Foraging Theory. Princeton: Princeton University Press.

Stewart, J. (1986). Calculus. Wandsworth: Belmont Inc.

Tolkamp, B. J. 1999. Limitations in the use of constraints for intake predictions. In: Regulation of feed intake. D. van der Heide *et al.* (Eds.) pp. 151-166. CAB International.

Tolkamp, B. J., D. J. Allcroft, E. J. Austin, B. L. Nielsen, and I. Kyriazakis. 1998a. Satiety splits feeding behaviour into bouts. Journal of Theoretical Biology 194:235-250.

Tolkamp, B. J., R. J. Dewhurst, N. C. Friggens, I. Kyriazakis, R. F. Veerkamp, and J. D. Oldham. 1998b. Diet choice by dairy cows. 1. Selection of feed protein content during the first half of lactation. Journal of Dairy Science 81:2657-2669.

Tolkamp, B. J., I. Kyriazakis, J. D. Oldham, M. Lewis, R. J. Dewhurst, and J. R. Newbold. 1998c. Diet choice by dairy cows. 2. Selection for metabolizable protein or for ruminally degradable protein? Journal of Dairy Science 81:2670-2680.

Tolkamp, B. J., N. C. Friggens, G. C. Emmans, I. Kyriazakis, and J. D. Oldham. 2002. Meal patterns of dairy cows consuming mixed foods with a high or a low ratio of concentrate to grass silage. Animal Science 74:369-382.

Tolkamp, B. J. and J. J. M. H. Ketelaars. 1992. Toward a new theory of feed intake regulation in ruminants. 2. Costs and benefits of feed consumption: an optimization approach. Livestock Production Science 30:297-317.

Tolkamp, B. J. and I. Kyriazakis. 1997. Measuring diet selection in dairy cows: effects of training on choice of dietary protein level. Animal Science 64:197-207.

Tolkamp, B. J. and I. Kyriazakis. 1999a. A comparison of five methods that estimate meal criteria for cattle. Animal Science 69:501-514.

Tolkamp, B. J. and I. Kyriazakis. 1999b. To split behaviour into bouts, log-transform the intervals. Animal Behaviour 57:807-817.

Tolkamp, B. J., D. P. N. Schweitzer, and I. Kyriazakis. 2000. The biologically relevant unit for the analysis of short-term feeding behavior of dairy cows. Journal of Dairy Science 83:2057-2068.

Weltner, K., Grosjean, J, Schuster, P. & Weber, W.J. (1986). Mathematics for Engineers and Scientists. Cheltenham: Stanley Thores Ltd.

Weston, R. 1996. Some aspects of constraint to forage consumption by ruminants. Australian Journal of Agricultural Research 47:175-197.

Weston, R. and D. Poppi. 1987. Comparitive aspects of food intake. In: Nutrition of Herbivores. pp. 133-161. Acedimic press Australia.

Whittemore, E. C., I. Kyriazakis, B. J. Tolkamp, and G. C. Emmans. 2002. The short-term feeding behavior of growing pigs fed foods differing in bulk content. Physiology and Behavior 76:131-141.

Winn, H. E., J. D. Goodyear, R. D. Kenney, and R. O. Petricig. 1995. Dive patterns of tagged right-whales in the Great South Channel. Continental Shelf Research 15:593-611.

Witt, M. W., L. A. Sinclair, R. G. Wilkinson, and P. J. Buttery. 1999a. The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on the metabolism and growth of ram lambs given food at a restricted level. Animal Science 69:627-636.

Witt, M. W., L. A. Sinclair, R. G. Wilkinson, and P. J. Buttery. 1999b. The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on the production and metabolism of sheep: food characterization and growth and metabolism of ewe lambs given food ad libitum. Animal Science 69:223-235.

Yearsley, J., B. J. Tolkamp, and A. W. Illius. 2001. Theoretical developments in the study and prediction of food intake. Proceedings of the Nutrition Society 60:145-156.

Zar, J. H. 1996. Biostatistical Analysis. Prentice-Hall International, New York.

APPENDIX A

Probability Density Functions:

Gaussian (**G**);

$$\text{pdf} = (1/(\sigma\sqrt{(2\pi)}))\exp(-(t-\mu)^2/2\sigma^2) \quad (1)$$

Weibull (**W**);

$$\text{pdf} = (c\, t^{(c-1)}/\alpha^c)\exp(-((t/\alpha)^c)) \quad (2)$$

Where t = $\log_e$(interval length in seconds); σ = standard deviations of the Gaussian distribution; μ = mean (median) of the Gaussian distribution; c = shape parameter of the Weibull distribution; α = scale parameter of the Weibull distribution. The median of the Weibull distribution = $\alpha(\log_e(2))^{1/c}$ (Johnson *et al.*, 1994).

These distributions can, for example, be combined into a three-population model. The first, second and third populations of intervals could therefore be described by Gaussian, Gaussian and Weibull distributions, respectively. Thus, a G-G-W model has a pdf as follows;

$$\begin{aligned}\text{pdf} = {} & p(1/(\sigma_1\sqrt{(2\pi)}))\exp(-(t-\mu_1)^2/2\sigma_1^2) + \\ & q\,(1/(\sigma_2\sqrt{(2\pi)}))\exp(-(t-\mu_2)^2/2\sigma_2^2) + \\ & (1-p-q)(c_3\, t^{(c_3-1)}/\alpha_3^{c_3})\exp(-((t/\alpha_3)^{c_3})) \end{aligned} \quad (3)$$

Where p = proportion of intervals in first population; q = proportion of intervals in the second population; Other parameters are as in (1) and (2) with subscripts $_1$, $_2$ & $_3$ indicating the first, second and third populations, respectively.

APPENDIX B

The original frequency distribution of bout composition was calculated for each experiment by randomly drawing each visit from the total population of visits. In an experiment with a proportion **p** of visits to H feeders, the probability that any randomly drawn visit is a visit to an H feeder or to an L feeder is then p and (1–p), respectively. In view of the propensity of cows to revisit the same feeder after being disturbed, the random probabilities of p and (1–p) are appropriate only for first visits in a meal. However, to calculate the random probabilities for all other visits (repeat visits) to an H feeder or an L feeder, the probabilities p and (1–p) have to be multiplied by the probability **u** that the repeat visit is not a result of disturbance. Therefore, the random probabilities of an animal repeating a visit to any H or L feeder are not p and (1-p) but (pu) and (1-p)u, respectively. Within all the experiments, the total numbers of H and L feeders were the same (**n** each). The random probability that an animal will revisit the same H feeder is then equal to $p \times pu/n$. This is the product of the probabilities that the previous visit is to an H feeder (p) and that the current visit is to the same H feeder [i.e. pu/n]. Similarly, the random probability of an animal immediately revisiting the same L feeder can be calculated as $(1-p) \times (1-p)u/n$. This is the product of the probabilities that the previous visit is to an L feeder (1-p) and that the current visit is to the same L feeder [i.e. (1-p)u/n]. By definition, revisits to the same feeder due to disturbance are a proportion of 1-u of all repeat visits. Therefore, revisits to the same feeder are a proportion **r** of all repeat visits [i.e. $r = p \times pu/n + (1-p) \times (1-p)u/n + 1-u$]. For any experiment, r can be

calculated by dividing the observed number of revisits to the same feeder by the number of repeat visits, i.e. the total number of visits minus the total number of meals (to exclude all first visits). Then r, p and n are known and the value for u can be calculated. For instance, for r = 0.3, p = 0.7 and n = 6, the value of u is 0.775. Now the probabilities for visits to H and L feeders occurring anywhere in a bout can be calculated from the equations in the following schedule.

Food type supplied by the feeder that was visited previously;	Probabilities of the current visit being to a feeder supplying;	
	H	L
None (i.e.: first visit of meal)	p	(1-p)
Different from current	pu	(1-p)u
Same as current	pu + (1–u)	(1-p)u + (1-u)

For instance, the probability of a visit to an H feeder is p = 0.7 only for first visits in a meal but the probabilities are pu = 0.5425 and pu + (1-u) = 0.7675 if the current visit follows a visit to an L or an H feeder, respectively. Similarly, the probability of a visit to an L feeder is (1-p) = 0.3 only for first visits in a meal but the probabilities are (1-p)u = 0.2325 and (1-p)u + (1-u) = 0.4575 if the current visit follows a visit to an H or an L feeder, respectively. This set of probabilities allows the calculation of the likelihood of any combination of visits in a bout. For instance, the probability that a bout of three visits consists of visits to, first, two L feeders and subsequently an H feeder is $0.3 \times 0.4575 \times 0.5425 = 0.0745$. This methodology was used to derive appropriate probabilities for each experiment. These probabilities were then used to calculate the corrected frequency distribution of bout composition.